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(54) Title: COMPOSITIONS FOR USE IN IDENTIFICATION OF BACTERIA

(57) Abstract: The present invention provides compositions, kits and methods for rapid identification and quantification of bacteria
by molecular mass and base composition analysis.

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COMPOSITIONS FOR USE IN IDENTIFICATION OF BACTERIA

STATEMENT OF GOVERNMENT SUPPORT

[01] This invention was made with United States Government support under CDC contract RO1 CI000099-01. The United States Government has certain rights in the invention.

FIELD OF THE INVENTION

[02] The present invention provides compositions, kits and methods for rapid identification and quantification of bacteria by molecular mass and base composition analysis.

BACKGROUND OF THE INVENTION

[03] A problem in determining the cause of a natural infectious outbreak or a bioterrorist attack is the sheer variety of organisms that can cause human disease. There are over 1400 organisms infectious to humans; many of these have the potential to emerge suddenly in a natural epidemic or to be used in a malicious attack by bioterrorists (Taylor et al. Philos. Trans. R. Soc. London B. Biol. Sci., 2001, 356, 983-989). This number does not include numerous strain variants, bioengineered versions, or pathogens that infect plants or animals.

[04] Much of the new technology being developed for detection of biological weapons incorporates a polymerase chain reaction (PCR) step based upon the use of highly specific primers and probes designed to selectively detect certain pathogenic organisms. Although this approach is appropriate for the most obvious bioterrorist organisms, like smallpox and anthrax, experience has shown that it is very difficult to predict which of hundreds of possible pathogenic organisms might be employed in a terrorist attack. Likewise, naturally emerging human disease that has caused devastating consequence in public health has come from unexpected families of bacteria, viruses, fungi, or protozoa. Plants and animals also have their natural burden of infectious disease agents and there are equally important biosafety and security concerns for agriculture.

[05] A major conundrum in public health protection, biodefense, and agricultural safety and security is that these disciplines need to be able to rapidly identify and characterize infectious agents, while there is no existing technology with the breadth of function to meet this need. Currently used methods for identification of bacteria rely upon culturing the bacterium to effect isolation from other

organisms and to obtain sufficient quantities of nucleic acid followed by sequencing of the nucleic acid, both processes which are time and labor intensive.

[06] Mass spectrometry provides detailed information about the molecules being analyzed, including high mass accuracy. It is also a process that can be easily automated. DNA chips with specific probes can only determine the presence or absence of specifically anticipated organisms. Because there are hundreds of thousands of species of benign bacteria, some very similar in sequence to threat organisms, even arrays with 10,000 probes lack the breadth needed to identify a particular organism.

[07] The present invention provides oligonucleotide primers and compositions and kits containing the oligonucleotide primers, which define bacterial bioagent identifying amplicons and, upon amplification, produce corresponding amplification products whose molecular masses provide the means to identify bacteria, for example, at and below the species taxonomic level.

SUMMARY OF THE INVENTION

[08] The present invention provides compositions, kits and methods for rapid identification and quantification of bacteria by molecular mass and base composition analysis.

[09] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456.

[10] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261.

[11] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261.

[12] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 288.

[13] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1269.

[14] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 288 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1269.

[15] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 698.

[16] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1420.

[17] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 698 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1420.

[18] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 217.

[19] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1167

[20] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 217 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1167.

[21] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 399.

[22] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1041.

[23] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 399 and an

oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1041.

[24] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 430.

[25] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1321.

[26] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 430 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1321.

[27] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 174.

[28] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 853.

[29] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 174 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 853.

[30] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 172.

[31] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1360.

[32] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 172 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1360.

[33] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261.

[34] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 456 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1261 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.

[35] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681.

[36] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022.

[37] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022.

[38] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 315.

[39] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1379.

[40] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 315 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1379.

[41] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 346.

[42] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 955.

[43] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 346 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 955.

[44] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 504.

[45] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1409.

[46] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 504 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1409.

[47] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 323.

[48] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1068.

[49] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 323 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1068.

[50] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 479.

[51] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 938.

[52] Another embodiment is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 479 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 938.

[53] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022.

[54] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 681 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1022 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 315:1379, 346:955, 504:1409, 323:1068, 479:938.

[55] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 583.

[56] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 923.

[57] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 583 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 923.

[58] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 454.

[59] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1418.

[60] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 454 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1418.

[61] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 250.

[62] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 902.

[63] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 250 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 902.

[64] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 384.

[65] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 878.

[66] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 384 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 878.

[67] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 694.

[68] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1215.

[69] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 694 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1215.

[70] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 194.

[71] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1173.

[72] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 194 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1173.

[73] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 375.

[74] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 890.

[75] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 375 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 890.

[76] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 656.

[77] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1224.

[78] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 656 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1224.

[79] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 618.

[80] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1157.

[81] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 618 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1157.

[82] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 302.

[83] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 852.

[84] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 302 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 852.

[85] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 199.

[86] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 889.

[87] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 199 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 889.

[88] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 596.

[89] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1169.

[90] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 596 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1169.

[91] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 150.

[92] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1242.

[93] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 150 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1242.

[94] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166.

[95] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1069.

[96] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1069.

[97] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166.

[98] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1168.

[99] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 166 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1168.

[100] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 583 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 923 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 454:1418, 250:902, 384:878, 694:1215, 194:1173, 375:890, 656:1224, 618:1157, 302:852, 199:889, 596:1169, 150:1242, 166:1069 and 166:1168.

[101] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 437.

[102] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1137.

[103] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 437 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1137.

[104] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 530.

[105] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 891.

[106] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 530 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 891.

[107] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 474.

[108] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 869.

[109] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 474 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 869.

[110] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 268.

[111] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1284.

[112] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 268 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1284.

[113] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 418.

[114] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1301.

[115] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 418 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1301.

- [116] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 318.
- [117] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1300.
- [118] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 318 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1300.
- [119] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 440.
- [120] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1076.
- [121] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 440 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1076.
- [122] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 219.
- [123] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1013.
- [124] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 219 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1013.
- [125] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 437 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence

identity with SEQ ID NO: 1137 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 530:891, 474:869, 268:1284, 418:1301, 318:1300, 440:1076 and 219:1013.

[126] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325.

[127] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163.

[128] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163.

[129] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 278.

[130] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1039.

[131] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 278 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1039.

[132] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 465.

[133] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1037.

[134] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 465 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1037.

[135] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 148.

[136] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1172.

[137] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 148 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1172.

[138] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 190.

[139] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1254.

[140] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 190 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1254.

[141] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 266.

[142] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1094.

[143] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 266 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1094.

[144] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 508.

[145] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1297.

[146] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 508 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1297.

[147] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 259.

[148] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1060.

[149] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 259 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1060.

[150] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 325 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1163 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 278:1039: 465:1037, 148:1172, 190:1254, 266:1094, 508:1297 and 259:1060.

[151] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376.

[152] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265.

[153] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265.

[154] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 267.

[155] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1341.

[156] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 267 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1341.

[157] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 705.

[158] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1056.

[159] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 705 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1056.

[160] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 710.

[161] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1259.

[162] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 710 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1259.

[163] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 374.

[164] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1111.

[165] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 374 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1111.

[166] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 545.

[167] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 978.

[168] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 545 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 978.

[169] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 249.

[170] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1095.

[171] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 249 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1095.

- [172] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 195.
- [173] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1376.
- [174] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 195 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1376.
- [175] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 311.
- [176] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1014.
- [177] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 311 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1014.
- [178] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 365.
- [179] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1052.
- [180] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 365 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1052.
- [181] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 527.

[182] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1071.

[183] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 527 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1071.

[184] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 490.

[185] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1182.

[186] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 490 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1182.

[187] Another embodiment is a kit comprising an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 376 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 1265 and further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 267:1341, 705:1056, 710:1259, 374:1111, 545:978, 249:1095, 195:1376, 311:1014, 365:1052, 527:1071 and 490:1182.

[188] In some embodiments, either or both of the primers of a primer pair composition contain at least one modified nucleobase such as 5-propynyluracil or 5-propynylcytosine for example.

[189] In some embodiments, either or both of the primers of the primer pair comprises at least one universal nucleobase such as inosine for example.

[190] In some embodiments, either or both of the primers of the primer pair comprises at least one non-templated T residue on the 5'-end.

[191] In some embodiments, either or both of the primers of the primer pair comprises at least one non-template tag.

[192] In some embodiments, either or both of the primers of the primer pair comprises at least one molecular mass modifying tag.

[193] In some embodiments, the present invention provides primers and compositions comprising pairs of primers, and kits containing the same, and methods for use in identification of bacteria. The primers are designed to produce amplification products of DNA encoding genes that have conserved and variable regions across different subgroups and genotypes of bacteria.

[194] Some embodiments are kits that contain one or more of the primer pair compositions. In some embodiments, each member of the one or more primer pairs of the kit is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from any of the primer pairs listed in Table 2.

[195] Some embodiments of the kits contain at least one calibration polynucleotide for use in quantitation of bacteria in a given sample, and also for use as a positive control for amplification.

[196] Some embodiments of the kits contain at least one anion exchange functional group linked to a magnetic bead.

[197] In some embodiments, the present invention also provides methods for identification of bacteria. Nucleic acid from the bacterium is amplified using the primers described above to obtain an amplification product. The molecular mass of the amplification product is measured. Optionally, the base composition of the amplification product is determined from the molecular mass. The molecular mass or base composition is compared with a plurality of molecular masses or base compositions of known analogous bacterial identifying amplicons, wherein a match between the molecular mass or base composition and a member of the plurality of molecular masses or base compositions identifies the bacterium. In some embodiments, the molecular mass is measured by mass spectrometry in a modality such as electrospray ionization (ESI) time of flight (TOF) mass spectrometry or ESI Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, for example. Other mass spectrometry techniques can also be used to measure the molecular mass of bacterial bioagent identifying amplicons.

[198] In some embodiments, the present invention is also directed to a method for determining the presence or absence of a bacterium in a sample. Nucleic acid from the sample is amplified using the composition described above to obtain an amplification product. The molecular mass of the amplification product is determined. Optionally, the base composition of the amplification product is determined from the molecular mass. The molecular mass or base composition of the amplification product is compared with the known molecular masses or base compositions of one or more known analogous bacterial bioagent identifying amplicons, wherein a match between the molecular mass or base composition of the amplification product and the molecular mass or base composition of one or more known bacterial bioagent identifying amplicons indicates the presence of the bacterium in the sample. In some embodiments, the molecular mass is measured by mass spectrometry.

[199] In some embodiments, the present invention also provides methods for determination of the quantity of an unknown bacterium in a sample. The sample is contacted with the composition described above and a known quantity of a calibration polynucleotide comprising a calibration sequence. Nucleic acid from the unknown bacterium in the sample is concurrently amplified with the composition described above and nucleic acid from the calibration polynucleotide in the sample is concurrently amplified with the composition described above to obtain a first amplification product comprising a bacterial bioagent identifying amplicon and a second amplification product comprising a calibration amplicon. The molecular masses and abundances for the bacterial bioagent identifying amplicon and the calibration amplicon are determined. The bacterial bioagent identifying amplicon is distinguished from the calibration amplicon based on molecular mass and comparison of bacterial bioagent identifying amplicon abundance and calibration amplicon abundance indicates the quantity of bacterium in the sample. In some embodiments, the base composition of the bacterial bioagent identifying amplicon is determined.

[200] In some embodiments, the present invention provides methods for detecting or quantifying bacteria by combining a nucleic acid amplification process with a mass determination process. In some embodiments, such methods identify or otherwise analyze the bacterium by comparing mass information from an amplification product with a calibration or control product. Such methods can be carried out in a highly multiplexed and/or parallel manner allowing for the analysis of as many as 300 samples per 24 hours on a single mass measurement platform. The accuracy of the mass determination methods in some embodiments of the present invention permits allows for the ability to discriminate between different bacteria such as, for example, various genotypes and drug resistant strains of *Staphylococcus aureus*.

BRIEF DESCRIPTION OF THE DRAWINGS

[201] The foregoing summary of the invention, as well as the following detailed description of the invention, is better understood when read in conjunction with the accompanying drawings which are included by way of example and not by way of limitation.

[202] **Figure 1:** process diagram illustrating a representative primer pair selection process.

[203] **Figure 2:** process diagram illustrating an embodiment of the calibration method.

[204] **Figure 3:** common pathogenic bacteria and primer pair coverage. The primer pair number in the upper right hand corner of each polygon indicates that the primer pair can produce a bioagent identifying amplicon for all species within that polygon.

[205] **Figure 4:** a representative 3D diagram of base composition (axes A, G and C) of bioagent identifying amplicons obtained with primer pair number 14 (a precursor of primer pair number 348 which targets 16S rRNA). The diagram indicates that the experimentally determined base compositions of the clinical samples (labeled NHRC samples) closely match the base compositions expected for *Streptococcus pyogenes* and are distinct from the expected base compositions of other organisms.

[206] **Figure 5:** a representative mass spectrum of amplification products indicating the presence of bioagent identifying amplicons of *Streptococcus pyogenes*, *Neisseria meningitidis*, and *Haemophilus influenzae* obtained from amplification of nucleic acid from a clinical sample with primer pair number 349 which targets 23S rRNA. Experimentally determined molecular masses and base compositions for the sense strand of each amplification product are shown.

[207] **Figure 6:** a representative mass spectrum of amplification products representing a bioagent identifying amplicon of *Streptococcus pyogenes*, and a calibration amplicon obtained from amplification of nucleic acid from a clinical sample with primer pair number 356 which targets rplB. The experimentally determined molecular mass and base composition for the sense strand of the *Streptococcus pyogenes* amplification product is shown.

[208] **Figure 7:** a representative mass spectrum of an amplified nucleic acid mixture which contained the Ames strain of *Bacillus anthracis*, a known quantity of combination calibration polynucleotide (SEQ ID NO: 1464), and primer pair number 350 which targets the capC gene on

the virulence plasmid pX02 of *Bacillus anthracis*. Calibration amplicons produced in the amplification reaction are visible in the mass spectrum as indicated and abundance data (peak height) are used to calculate the quantity of the Ames strain of *Bacillus anthracis*.

DEFINITIONS

[209] As used herein, the term "abundance" refers to an amount. The amount may be described in terms of concentration which are common in molecular biology such as "copy number," "pfu or plate-forming unit" which are well known to those with ordinary skill. Concentration may be relative to a known standard or may be absolute.

[210] As used herein, the term "amplifiable nucleic acid" is used in reference to nucleic acids that may be amplified by any amplification method. It is contemplated that "amplifiable nucleic acid" also comprises "sample template."

[211] As used herein the term "amplification" refers to a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (i.e., replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (i.e., synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out. Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Q β replicase, MDV-1 RNA is the specific template for the replicase (D.L. Kacian et al., Proc. Natl. Acad. Sci. USA 69:3038 [1972]). Other nucleic acid will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters (Chamberlin et al., Nature 228:227 [1970]). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction (D.Y. Wu and R. B. Wallace, Genomics 4:560 [1989]). Finally, Taq and Pfu polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences (H.A. Erlich (ed.), PCR Technology, Stockton Press [1989]).

[212] As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification, excluding primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

[213] As used herein, the term "analogous" when used in context of comparison of bioagent identifying amplicons indicates that the bioagent identifying amplicons being compared are produced with the same pair of primers. For example, bioagent identifying amplicon "A" and bioagent identifying amplicon "B", produced with the same pair of primers are analogous with respect to each other. Bioagent identifying amplicon "C", produced with a different pair of primers is not analogous to either bioagent identifying amplicon "A" or bioagent identifying amplicon "B".

[214] As used herein, the term "anion exchange functional group" refers to a positively charged functional group capable of binding an anion through an electrostatic interaction. The most well known anion exchange functional groups are the amines, including primary, secondary, tertiary and quaternary amines.

[215] The term "bacteria" or "bacterium" refers to any member of the groups of eubacteria and archaeobacteria.

[216] As used herein, a "base composition" is the exact number of each nucleobase (for example, A, T, C and G) in a segment of nucleic acid. For example, amplification of nucleic acid of *Staphylococcus aureus* strain carrying the lukS-PV gene with primer pair number 2095 (SEQ ID NOs: 456:1261) produces an amplification product 117 nucleobases in length from nucleic acid of the lukS-PV gene that has a base composition of A35 G17 C19 T46 (by convention - with reference to the sense strand of the amplification product). Because the molecular masses of each of the four natural nucleotides and chemical modifications thereof are known (if applicable), a measured molecular mass can be deconvoluted to a list of possible base compositions. Identification of a base composition of a sense strand which is complementary to the corresponding antisense strand in terms of base composition provides a confirmation of the true base composition of an unknown amplification product. For example, the base composition of the antisense strand of the 139 nucleobase amplification product described above is A46 G19 C17 T35.

[217] As used herein, a "base composition probability cloud" is a representation of the diversity in base composition resulting from a variation in sequence that occurs among different isolates of a given

species. The "base composition probability cloud" represents the base composition constraints for each species and is typically visualized using a pseudo four-dimensional plot.

[218] In the context of this invention, a "bioagent" is any organism, cell, or virus, living or dead, or a nucleic acid derived from such an organism, cell or virus. Examples of bioagents include, but are not limited, to cells, (including but not limited to human clinical samples, bacterial cells and other pathogens), viruses, fungi, protists, parasites, and pathogenicity markers (including but not limited to: pathogenicity islands, antibiotic resistance genes, virulence factors, toxin genes and other bioregulating compounds). Samples may be alive or dead or in a vegetative state (for example, vegetative bacteria or spores) and may be encapsulated or bioengineered. In the context of this invention, a "pathogen" is a bioagent which causes a disease or disorder.

[219] As used herein, a "bioagent division" is defined as group of bioagents above the species level and includes but is not limited to, orders, families, classes, clades, genera or other such groupings of bioagents above the species level.

[220] As used herein, the term "bioagent identifying amplicon" refers to a polynucleotide that is amplified from a bioagent in an amplification reaction and which 1) provides sufficient variability to distinguish among bioagents from whose nucleic acid the bioagent identifying amplicon is produced and 2) whose molecular mass is amenable to a rapid and convenient molecular mass determination modality such as mass spectrometry, for example.

[221] As used herein, the term "biological product" refers to any product originating from an organism. Biological products are often products of processes of biotechnology. Examples of biological products include, but are not limited to: cultured cell lines, cellular components, antibodies, proteins and other cell-derived biomolecules, growth media, growth harvest fluids, natural products and biopharmaceutical products.

[222] The terms "biowarfare agent" and "bioweapon" are synonymous and refer to a bacterium, virus, fungus or protozoan that could be deployed as a weapon to cause bodily harm to individuals. Military or terrorist groups may be implicated in deployment of biowarfare agents.

[223] In context of this invention, the term "broad range survey primer pair" refers to a primer pair designed to produce bioagent identifying amplicons across different broad groupings of bioagents. For example, the ribosomal RNA-targeted primer pairs are broad range survey primer pairs which have the capability of producing bacterial bioagent identifying amplicons for essentially all known bacteria. With

respect to broad range primer pairs employed for identification of bacteria, a broad range survey primer pair for bacteria such as 16S rRNA primer pair number 346 (SEQ ID NOs: 202:1110) for example, will produce an bacterial bioagent identifying amplicon for essentially all known bacteria.

[224] The term "calibration amplicon" refers to a nucleic acid segment representing an amplification product obtained by amplification of a calibration sequence with a pair of primers designed to produce a bioagent identifying amplicon.

[225] The term "calibration sequence" refers to a polynucleotide sequence to which a given pair of primers hybridizes for the purpose of producing an internal (i.e: included in the reaction) calibration standard amplification product for use in determining the quantity of a bioagent in a sample. The calibration sequence may be expressly added to an amplification reaction, or may already be present in the sample prior to analysis.

[226] The term "clade primer pair" refers to a primer pair designed to produce bioagent identifying amplicons for species belonging to a clade group. A clade primer pair may also be considered as a "speciating" primer pair which is useful for distinguishing among closely related species.

[227] The term "codon" refers to a set of three adjoined nucleotides (triplet) that codes for an amino acid or a termination signal.

[228] In context of this invention, the term "codon base composition analysis," refers to determination of the base composition of an individual codon by obtaining a bioagent identifying amplicon that includes the codon. The bioagent identifying amplicon will at least include regions of the target nucleic acid sequence to which the primers hybridize for generation of the bioagent identifying amplicon as well as the codon being analyzed, located between the two primer hybridization regions.

[229] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides such as an oligonucleotide or a target nucleic acid) related by the base-pairing rules. For example, for the sequence "5'-A-G-T-3'," is complementary to the sequence "3'-T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids. Either term may also be used in reference to individual nucleotides, especially within the

context of polynucleotides. For example, a particular nucleotide within an oligonucleotide may be noted for its complementarity, or lack thereof, to a nucleotide within another nucleic acid strand, in contrast or comparison to the complementarity between the rest of the oligonucleotide and the nucleic acid strand.

[230] The term "complement of a nucleic acid sequence" as used herein refers to an oligonucleotide which, when aligned with the nucleic acid sequence such that the 5' end of one sequence is paired with the 3' end of the other, is in "antiparallel association." Certain bases not commonly found in natural nucleic acids may be included in the nucleic acids of the present invention and include, for example, inosine and 7-deazaguanine. Complementarity need not be perfect; stable duplexes may contain mismatched base pairs or unmatched bases. Those skilled in the art of nucleic acid technology can determine duplex stability empirically considering a number of variables including, for example, the length of the oligonucleotide, base composition and sequence of the oligonucleotide, ionic strength and incidence of mismatched base pairs. Where a first oligonucleotide is complementary to a region of a target nucleic acid and a second oligonucleotide has complementary to the same region (or a portion of this region) a "region of overlap" exists along the target nucleic acid. The degree of overlap will vary depending upon the extent of the complementarity.

[231] In context of this invention, the term "division-wide primer pair" refers to a primer pair designed to produce bioagent identifying amplicons within sections of a broader spectrum of bioagents. For example, primer pair number 352 (SEQ ID NOs: 687:1411), a division-wide primer pair, is designed to produce bacterial bioagent identifying amplicons for members of the *Bacillus* group of bacteria which comprises, for example, members of the genera *Streptococci*, *Enterococci*, and *Staphylococci*. Other division-wide primer pairs may be used to produce bacterial bioagent identifying amplicons for other groups of bacterial bioagents.

[232] As used herein, the term "concurrently amplifying" used with respect to more than one amplification reaction refers to the act of simultaneously amplifying more than one nucleic acid in a single reaction mixture.

[233] As used herein, the term "drill-down primer pair" refers to a primer pair designed to produce bioagent identifying amplicons for identification of sub-species characteristics or confirmation of a species assignment. For example, primer pair number 2146 (SEQ ID NOs: 437:1137), a drill-down *Staphylococcus aureus* genotyping primer pair, is designed to produce *Staphylococcus aureus* genotyping amplicons. Other drill-down primer pairs may be used to produce bioagent identifying amplicons for *Staphylococcus aureus* and other bacterial species.

[234] The term "duplex" refers to the state of nucleic acids in which the base portions of the nucleotides on one strand are bound through hydrogen bonding to their complementary bases arrayed on a second strand. The condition of being in a duplex form reflects on the state of the bases of a nucleic acid. By virtue of base pairing, the strands of nucleic acid also generally assume the tertiary structure of a double helix, having a major and a minor groove. The assumption of the helical form is implicit in the act of becoming duplexed.

[235] As used herein, the term "etiology" refers to the causes or origins, of diseases or abnormal physiological conditions.

[236] The term "gene" refers to a DNA sequence that comprises control and coding sequences necessary for the production of an RNA having a non-coding function (e.g., a ribosomal or transfer RNA), a polypeptide or a precursor. The RNA or polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or function is retained.

[237] The terms "homology," "homologous" and "sequence identity" refer to a degree of identity. There may be partial homology or complete homology. A partially homologous sequence is one that is less than 100% identical to another sequence. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is otherwise identical to another 20 nucleobase primer but having two non-identical residues has 18 of 20 identical residues ($18/20 = 0.9$ or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of a primer 20 nucleobases in length would have $15/20 = 0.75$ or 75% sequence identity with the 20 nucleobase primer. In context of the present invention, sequence identity is meant to be properly determined when the query sequence and the subject sequence are both described and aligned in the 5' to 3' direction. Sequence alignment algorithms such as BLAST, will return results in two different alignment orientations. In the Plus/Plus orientation, both the query sequence and the subject sequence are aligned in the 5' to 3' direction. On the other hand, in the Plus/Minus orientation, the query sequence is in the 5' to 3' direction while the subject sequence is in the 3' to 5' direction. It should be understood that with respect to the primers of the present invention, sequence identity is properly determined when the alignment is designated as Plus/Plus. Sequence identity may also encompass alternate or modified nucleobases that perform in a functionally similar manner to the regular nucleobases adenine, thymine, guanine and cytosine with respect to hybridization and primer extension in amplification reactions. In a non-limiting example, if the 5-propynyl pyrimidines propyne C and/or propyne T replace one or more C or T residues in one primer which is otherwise identical to another primer in sequence and length, the two primers will have 100% sequence identity with each other. In another non-limiting example, Inosine (I) may be used as a replacement for G or T and effectively

hybridize to C, A or U (uracil). Thus, if inosine replaces one or more C, A or U residues in one primer which is otherwise identical to another primer in sequence and length, the two primers will have 100% sequence identity with each other. Other such modified or universal bases may exist which would perform in a functionally similar manner for hybridization and amplification reactions and will be understood to fall within this definition of sequence identity.

[238] As used herein, "housekeeping gene" refers to a gene encoding a protein or RNA involved in basic functions required for survival and reproduction of a bioagent. Housekeeping genes include, but are not limited to genes encoding RNA or proteins involved in translation, replication, recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, secretion and the like.

[239] As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is influenced by such factors as the degree of complementarity between the nucleic acids, stringency of the conditions involved, and the T_m of the formed hybrid. "Hybridization" methods involve the annealing of one nucleic acid to another, complementary nucleic acid, i.e., a nucleic acid having a complementary nucleotide sequence. The ability of two polymers of nucleic acid containing complementary sequences to find each other and anneal through base pairing interaction is a well-recognized phenomenon. The initial observations of the "hybridization" process by Marmur and Lane, Proc. Natl. Acad. Sci. USA 46:453 (1960) and Doty et al., Proc. Natl. Acad. Sci. USA 46:461 (1960) have been followed by the refinement of this process into an essential tool of modern biology.

[240] The term "*in silico*" refers to processes taking place via computer calculations. For example, electronic PCR (ePCR) is a process analogous to ordinary PCR except that it is carried out using nucleic acid sequences and primer pair sequences stored on a computer formatted medium.

[241] As used herein, "intelligent primers" are primers that are designed to bind to highly conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and, upon amplification, yield amplification products which ideally provide enough variability to distinguish individual bioagents, and which are amenable to molecular mass analysis. By the term "highly conserved," it is meant that the sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity among all, or at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% of species or strains.

[242] The "ligase chain reaction" (LCR; sometimes referred to as "Ligase Amplification Reaction" (LAR) described by Barany, *Proc. Natl. Acad. Sci.*, 88:189 (1991); Barany, *PCR Methods and Applic.*, 1:5 (1991); and Wu and Wallace, *Genomics* 4:560 (1989) has developed into a well-recognized alternative method for amplifying nucleic acids. In LCR, four oligonucleotides, two adjacent oligonucleotides which uniquely hybridize to one strand of target DNA, and a complementary set of adjacent oligonucleotides, that hybridize to the opposite strand are mixed and DNA ligase is added to the mixture. Provided that there is complete complementarity at the junction, ligase will covalently link each set of hybridized molecules. Importantly, in LCR, two probes are ligated together only when they base-pair with sequences in the target sample, without gaps or mismatches. Repeated cycles of denaturation, hybridization and ligation amplify a short segment of DNA. LCR has also been used in combination with PCR to achieve enhanced detection of single-base changes. However, because the four oligonucleotides used in this assay can pair to form two short ligatable fragments, there is the potential for the generation of target-independent background signal. The use of LCR for mutant screening is limited to the examination of specific nucleic acid positions.

[243] The term "locked nucleic acid" or "LNA" refers to a nucleic acid analogue containing one or more 2'-O, 4'-C-methylene- β -D-ribofuranosyl nucleotide monomers in an RNA mimicking sugar conformation. LNA oligonucleotides display unprecedented hybridization affinity toward complementary single-stranded RNA and complementary single- or double-stranded DNA. LNA oligonucleotides induce A-type (RNA-like) duplex conformations. The primers of the present invention may contain LNA modifications.

[244] As used herein, the term "mass-modifying tag" refers to any modification to a given nucleotide which results in an increase in mass relative to the analogous non-mass modified nucleotide. Mass-modifying tags can include heavy isotopes of one or more elements included in the nucleotide such as carbon-13 for example. Other possible modifications include addition of substituents such as iodine or bromine at the 5 position of the nucleobase for example.

[245] The term "mass spectrometry" refers to measurement of the mass of atoms or molecules. The molecules are first converted to ions, which are separated using electric or magnetic fields according to the ratio of their mass to electric charge. The measured masses are used to identify the molecules.

[246] The term "microorganism" as used herein means an organism too small to be observed with the unaided eye and includes, but is not limited to bacteria, virus, protozoans, fungi; and ciliates.

[247] The term "multi-drug resistant" or multiple-drug resistant" refers to a microorganism which is resistant to more than one of the antibiotics or antimicrobial agents used in the treatment of said microorganism.

[248] The term "multiplex PCR" refers to a PCR reaction where more than one primer set is included in the reaction pool allowing 2 or more different DNA targets to be amplified by PCR in a single reaction tube.

[249] The term "non-template tag" refers to a stretch of at least three guanine or cytosine nucleobases of a primer used to produce a bioagent identifying amplicon which are not complementary to the template. A non-template tag is incorporated into a primer for the purpose of increasing the primer-duplex stability of later cycles of amplification by incorporation of extra G-C pairs which each have one additional hydrogen bond relative to an A-T pair.

[250] The term "nucleic acid sequence" as used herein refers to the linear composition of the nucleic acid residues A, T, C or G or any modifications thereof, within an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single or double stranded, and represent the sense or antisense strand

[251] As used herein, the term "nucleobase" is synonymous with other terms in use in the art including "nucleotide," "deoxynucleotide," "nucleotide residue," "deoxynucleotide residue," "nucleotide triphosphate (NTP)," or deoxynucleotide triphosphate (dNTP).

[252] The term "nucleotide analog" as used herein refers to modified or non-naturally occurring nucleotides such as 5-propynyl pyrimidines (i.e., 5-propynyl-dTTP and 5-propynyl-dTCP), 7-deaza purines (i.e., 7-deaza-dATP and 7-deaza-dGTP). Nucleotide analogs include base analogs and comprise modified forms of deoxyribonucleotides as well as ribonucleotides.

[253] The term "oligonucleotide" as used herein is defined as a molecule comprising two or more deoxyribonucleotides or ribonucleotides, preferably at least 5 nucleotides, more preferably at least about 13 to 35 nucleotides. The exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. The oligonucleotide may be generated in any manner, including chemical synthesis, DNA replication, reverse transcription, PCR, or a combination thereof. Because mononucleotides are reacted to make oligonucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage, an end of an oligonucleotide is referred to as the "5'-end" if its 5' phosphate is not

linked to the 3' oxygen of a mononucleotide pentose ring and as the "3'-end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide, also may be said to have 5' and 3' ends. A first region along a nucleic acid strand is said to be upstream of another region if the 3' end of the first region is before the 5' end of the second region when moving along a strand of nucleic acid in a 5' to 3' direction. All oligonucleotide primers disclosed herein are understood to be presented in the 5' to 3' direction when reading left to right. When two different, non-overlapping oligonucleotides anneal to different regions of the same linear complementary nucleic acid sequence, and the 3' end of one oligonucleotide points towards the 5' end of the other, the former may be called the "upstream" oligonucleotide and the latter the "downstream" oligonucleotide. Similarly, when two overlapping oligonucleotides are hybridized to the same linear complementary nucleic acid sequence, with the first oligonucleotide positioned such that its 5' end is upstream of the 5' end of the second oligonucleotide, and the 3' end of the first oligonucleotide is upstream of the 3' end of the second oligonucleotide, the first oligonucleotide may be called the "upstream" oligonucleotide and the second oligonucleotide may be called the "downstream" oligonucleotide.

[254] In the context of this invention, a "pathogen" is a bioagent which causes a disease or disorder.

[255] As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

[256] The term "peptide nucleic acid" ("PNA") as used herein refers to a molecule comprising bases or base analogs such as would be found in natural nucleic acid, but attached to a peptide backbone rather than the sugar-phosphate backbone typical of nucleic acids. The attachment of the bases to the peptide is such as to allow the bases to base pair with complementary bases of nucleic acid in a manner similar to that of an oligonucleotide. These small molecules, also designated anti gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, et al. *Anticancer Drug Des.* 8:53-63). The primers of the present invention may comprise PNAs.

[257] The term "polymerase" refers to an enzyme having the ability to synthesize a complementary strand of nucleic acid from a starting template nucleic acid strand and free dNTPs.

[258] As used herein, the term "polymerase chain reaction" ("PCR") refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, hereby incorporated by reference, that

describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (i.e., denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified." With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (e.g., hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ^{32}P -labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

[259] The term "polymerization means" or "polymerization agent" refers to any agent capable of facilitating the addition of nucleoside triphosphates to an oligonucleotide. Preferred polymerization means comprise DNA and RNA polymerases.

[260] As used herein, the terms "pair of primers," or "primer pair" are synonymous. A primer pair is used for amplification of a nucleic acid sequence. A pair of primers comprises a forward primer and a reverse primer. The forward primer hybridizes to a sense strand of a target gene sequence to be amplified and primes synthesis of an antisense strand (complementary to the sense strand) using the target sequence as a template. A reverse primer hybridizes to the antisense strand of a target gene sequence to be amplified and primes synthesis of a sense strand (complementary to the antisense strand) using the target sequence as a template.

[261] The primers are designed to bind to highly conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and yield amplification products which ideally provide enough variability to distinguish each individual bioagent, and which are amenable to molecular mass analysis. In some embodiments, the highly conserved sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity, or between about 99-100% identity. The molecular mass of a given amplification product provides a means of identifying the bioagent from which it was obtained, due to the variability of the variable region. Thus design of the primers requires selection of a variable region with appropriate variability to resolve the identity of a given bioagent. Bioagent identifying amplicons are ideally specific to the identity of the bioagent.

[262] Properties of the primers may include any number of properties related to structure including, but not limited to: nucleobase length which may be contiguous (linked together) or non-contiguous (for example, two or more contiguous segments which are joined by a linker or loop moiety), modified or universal nucleobases (used for specific purposes such as for example, increasing hybridization affinity, preventing non-templated adenylation and modifying molecular mass) percent complementarity to a given target sequences.

[263] Properties of the primers also include functional features including, but not limited to, orientation of hybridization (forward or reverse) relative to a nucleic acid template. The coding or sense strand is the strand to which the forward priming primer hybridizes (forward priming orientation) while the reverse priming primer hybridizes to the non-coding or antisense strand (reverse priming orientation). The functional properties of a given primer pair also include the generic template nucleic acid to which the primer pair hybridizes. For example, identification of bioagents can be accomplished at different levels using primers suited to resolution of each individual level of identification. Broad range survey primers are designed with the objective of identifying a bioagent as a member of a particular division (e.g., an order, family, genus or other such grouping of bioagents above the species level of bioagents). In some embodiments, broad range survey intelligent primers are capable of identification of bioagents at the species or sub-species level. Other primers may have the functionality of producing bioagent identifying amplicons for members of a given taxonomic genus, clade, species, sub-species or genotype (including genetic variants which may include presence of virulence genes or antibiotic resistance genes or mutations). Additional functional properties of primer pairs include the functionality of performing amplification either singly (single primer pair per amplification reaction vessel) or in a multiplex fashion (multiple primer pairs and multiple amplification reactions within a single reaction vessel).

[264] As used herein, the terms "purified" or "substantially purified" refer to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated,

and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated. An "isolated polynucleotide" or "isolated oligonucleotide" is therefore a substantially purified polynucleotide.

[265] The term "reverse transcriptase" refers to an enzyme having the ability to transcribe DNA from an RNA template. This enzymatic activity is known as reverse transcriptase activity. Reverse transcriptase activity is desirable in order to obtain DNA from RNA viruses which can then be amplified and analyzed by the methods of the present invention.

[266] The term "ribosomal RNA" or "rRNA" refers to the primary ribonucleic acid constituent of ribosomes. Ribosomes are the protein-manufacturing organelles of cells and exist in the cytoplasm. Ribosomal RNAs are transcribed from the DNA genes encoding them.

[267] The term "sample" in the present specification and claims is used in its broadest sense. On the one hand it is meant to include a specimen or culture (e.g., microbiological cultures). On the other hand, it is meant to include both biological and environmental samples. A sample may include a specimen of synthetic origin. Biological samples may be animal, including human, fluid, solid (e.g., stool) or tissue, as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Biological samples may be obtained from all of the various families of domestic animals, as well as feral or wild animals, including, but not limited to, such animals as ungulates, bear, fish, lagomorphs, rodents, etc. Environmental samples include environmental material such as surface matter, soil, water, air and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items. These examples are not to be construed as limiting the sample types applicable to the present invention. The term "source of target nucleic acid" refers to any sample that contains nucleic acids (RNA or DNA). Particularly preferred sources of target nucleic acids are biological samples including, but not limited to blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum and semen.

[268] As used herein, the term "sample template" refers to nucleic acid originating from a sample that is analyzed for the presence of "target" (defined below). In contrast, "background template" is used in reference to nucleic acid other than sample template that may or may not be present in a sample. Background template is often a contaminant. It may be the result of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

[269] A "segment" is defined herein as a region of nucleic acid within a target sequence.

[270] The "self-sustained sequence replication reaction" (3SR) (Guatelli et al., Proc. Natl. Acad. Sci., 87:1874-1878 [1990], with an erratum at Proc. Natl. Acad. Sci., 87:7797 [1990]) is a transcription-based *in vitro* amplification system (Kwok et al., Proc. Natl. Acad. Sci., 86:1173-1177 [1989]) that can exponentially amplify RNA sequences at a uniform temperature. The amplified RNA can then be utilized for mutation detection (Fahy et al., PCR Meth. Appl., 1:25-33 [1991]). In this method, an oligonucleotide primer is used to add a phage RNA polymerase promoter to the 5' end of the sequence of interest. In a cocktail of enzymes and substrates that includes a second primer, reverse transcriptase, RNase H, RNA polymerase and ribo- and deoxyribonucleoside triphosphates, the target sequence undergoes repeated rounds of transcription, cDNA synthesis and second-strand synthesis to amplify the area of interest. The use of 3SR to detect mutations is kinetically limited to screening small segments of DNA (e.g., 200-300 base pairs).

[271] As used herein, the term "sequence alignment" refers to a listing of multiple DNA or amino acid sequences and aligns them to highlight their similarities. The listings can be made using bioinformatics computer programs.

[272] In context of this invention, the term "speciating primer pair" refers to a primer pair designed to produce a bioagent identifying amplicon with the diagnostic capability of identifying species members of a group of genera or a particular genus of bioagents. Primer pair number 2249 (SEQ ID NOs: 430:1321), for example, is a speciating primer pair used to distinguish *Staphylococcus aureus* from other species of the genus *Staphylococcus*.

[273] As used herein, a "sub-species characteristic" is a genetic characteristic that provides the means to distinguish two members of the same bioagent species. For example, one viral strain could be distinguished from another viral strain of the same species by possessing a genetic change (e.g., for example, a nucleotide deletion, addition or substitution) in one of the viral genes, such as the RNA-dependent RNA polymerase. Sub-species characteristics such as virulence genes and drug-resistance are responsible for the phenotypic differences among the different strains of bacteria.

[274] As used herein, the term "target" is used in a broad sense to indicate the gene or genomic region being amplified by the primers. Because the present invention provides a plurality of amplification products from any given primer pair (depending on the bioagent being analyzed), multiple amplification products from different specific nucleic acid sequences may be obtained. Thus, the term "target" is not used to refer to a single specific nucleic acid sequence. The "target" is sought to be sorted out from other nucleic acid sequences and contains a sequence that has at least partial complementarity with an

oligonucleotide primer. The target nucleic acid may comprise single- or double-stranded DNA or RNA. A "segment" is defined as a region of nucleic acid within the target sequence.

[275] The term "template" refers to a strand of nucleic acid on which a complementary copy is built from nucleoside triphosphates through the activity of a template-dependent nucleic acid polymerase. Within a duplex the template strand is, by convention, depicted and described as the "bottom" strand. Similarly, the non-template strand is often depicted and described as the "top" strand.

[276] As used herein, the term " T_m " is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% \text{ G+C})$, when a nucleic acid is in aqueous solution at 1 M NaCl (see e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985). Other references (e.g., Allawi, H. T. & SantaLucia, J., Jr. Thermodynamics and NMR of internal G.T mismatches in DNA. Biochemistry 36, 10581-94 (1997) include more sophisticated computations which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m .

[277] The term "triangulation genotyping analysis" refers to a method of genotyping a bioagent by measurement of molecular masses or base compositions of amplification products, corresponding to bioagent identifying amplicons, obtained by amplification of regions of more than one gene. In this sense, the term "triangulation" refers to a method of establishing the accuracy of information by comparing three or more types of independent points of view bearing on the same findings. Triangulation genotyping analysis carried out with a plurality of triangulation genotyping analysis primers yields a plurality of base compositions that then provide a pattern or "barcode" from which a species type can be assigned. The species type may represent a previously known sub-species or strain, or may be a previously unknown strain having a specific and previously unobserved base composition barcode indicating the existence of a previously unknown genotype.

[278] As used herein, the term "triangulation genotyping analysis primer pair" is a primer pair designed to produce bioagent identifying amplicons for determining species types in a triangulation genotyping analysis.

[279] The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as "triangulation identification." Triangulation identification is pursued by

analyzing a plurality of bioagent identifying amplicons produced with different primer pairs. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical of *B. anthracis* (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the *B. anthracis* genome would suggest a genetic engineering event.

[280] In the context of this invention, the term "unknown bioagent" may mean either: (i) a bioagent whose existence is known (such as the well known bacterial species *Staphylococcus aureus* for example) but which is not known to be in a sample to be analyzed, or (ii) a bioagent whose existence is not known (for example, the SARS coronavirus was unknown prior to April 2003). For example, if the method for identification of coronaviruses disclosed in commonly owned U.S. Patent Serial No. 10/829,826 (incorporated herein by reference in its entirety) was to be employed prior to April 2003 to identify the SARS coronavirus in a clinical sample, both meanings of "unknown" bioagent are applicable since the SARS coronavirus was unknown to science prior to April, 2003 and since it was not known what bioagent (in this case a coronavirus) was present in the sample. On the other hand, if the method of U.S. Patent Serial No. 10/829,826 was to be employed subsequent to April 2003 to identify the SARS coronavirus in a clinical sample, only the first meaning (i) of "unknown" bioagent would apply since the SARS coronavirus became known to science subsequent to April 2003 and since it was not known what bioagent was present in the sample.

[281] The term "variable sequence" as used herein refers to differences in nucleic acid sequence between two nucleic acids. For example, the genes of two different bacterial species may vary in sequence by the presence of single base substitutions and/or deletions or insertions of one or more nucleotides. These two forms of the structural gene are said to vary in sequence from one another. In the context of the present invention, "viral nucleic acid" includes, but is not limited to, DNA, RNA, or DNA that has been obtained from viral RNA, such as, for example, by performing a reverse transcription reaction. Viral RNA can either be single-stranded (of positive or negative polarity) or double-stranded.

[282] The term "virus" refers to obligate, ultramicroscopic, parasites that are incapable of autonomous replication (i.e., replication requires the use of the host cell's machinery). Viruses can survive outside of a host cell but cannot replicate.

[283] The term "wild-type" refers to a gene or a gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified", "mutant" or "polymorphic" refers to a gene or gene product

that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

[284] As used herein, a “wobble base” is a variation in a codon found at the third nucleotide position of a DNA triplet. Variations in conserved regions of sequence are often found at the third nucleotide position due to redundancy in the amino acid code.

DETAILED DESCRIPTION OF EMBODIMENTS

A. Bioagent Identifying Amplicons

[285] The present invention provides methods for detection and identification of unknown bioagents using bioagent identifying amplicons. Primers are selected to hybridize to conserved sequence regions of nucleic acids derived from a bioagent, and which bracket variable sequence regions to yield a bioagent identifying amplicon, which can be amplified and which is amenable to molecular mass determination. The molecular mass then provides a means to uniquely identify the bioagent without a requirement for prior knowledge of the possible identity of the bioagent. The molecular mass or corresponding base composition signature of the amplification product is then matched against a database of molecular masses or base composition signatures. A match is obtained when an experimentally-determined molecular mass or base composition of an analyzed amplification product is compared with known molecular masses or base compositions of known bioagent identifying amplicons and the experimentally determined molecular mass or base composition is the same as the molecular mass or base composition of one of the known bioagent identifying amplicons. Alternatively, the experimentally-determined molecular mass or base composition may be within experimental error of the molecular mass or base composition of a known bioagent identifying amplicon and still be classified as a match. In some cases, the match may also be classified using a probability of match model such as the models described in U.S. Serial No. 11/073,362, which is commonly owned and incorporated herein by reference in entirety. Furthermore, the method can be applied to rapid parallel multiplex analyses, the results of which can be employed in a triangulation identification strategy. The present method provides rapid throughput and does not require nucleic acid sequencing of the amplified target sequence for bioagent detection and identification.

[286] Despite enormous biological diversity, all forms of life on earth share sets of essential, common features in their genomes. Since genetic data provide the underlying basis for identification of bioagents by the methods of the present invention, it is necessary to select segments of nucleic acids which ideally provide enough variability to distinguish each individual bioagent and whose molecular mass is amenable to molecular mass determination.

[287] Unlike bacterial genomes, which exhibit conservation of numerous genes (i.e. housekeeping genes) across all organisms, viruses do not share a gene that is essential and conserved among all virus families. Therefore, viral identification is achieved within smaller groups of related viruses, such as members of a particular virus family or genus. For example, RNA-dependent RNA polymerase is present in all single-stranded RNA viruses and can be used for broad priming as well as resolution within the virus family.

[288] In some embodiments of the present invention, at least one bacterial nucleic acid segment is amplified in the process of identifying the bacterial bioagent. Thus, the nucleic acid segments that can be amplified by the primers disclosed herein and that provide enough variability to distinguish each individual bioagent and whose molecular masses are amenable to molecular mass determination are herein described as bioagent identifying amplicons.

[289] In some embodiments of the present invention, bioagent identifying amplicons comprise from about 45 to about 150 nucleobases (i.e. from about 45 to about 200 linked nucleosides), although both longer and short regions may be used. One of ordinary skill in the art will appreciate that the invention embodies compounds of 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150 nucleobases in length, or any range therewithin.

[290] It is the combination of the portions of the bioagent nucleic acid segment to which the primers hybridize (hybridization sites) and the variable region between the primer hybridization sites that comprises the bioagent identifying amplicon. Thus, it can be said that a given bioagent identifying amplicon is "defined by" a given pair of primers.

[291] In some embodiments, bioagent identifying amplicons amenable to molecular mass determination which are produced by the primers described herein are either of a length, size or mass compatible with the particular mode of molecular mass determination or compatible with a means of providing a predictable fragmentation pattern in order to obtain predictable fragments of a length compatible with the particular mode of molecular mass determination. Such means of providing a predictable fragmentation pattern of an amplification product include, but are not limited to, cleavage with chemical reagents, restriction enzymes or cleavage primers, for example. Thus, in some

embodiments, bioagent identifying amplicons are larger than 150 nucleobases and are amenable to molecular mass determination following restriction digestion. Methods of using restriction enzymes and cleavage primers are well known to those with ordinary skill in the art.

[292] In some embodiments, amplification products corresponding to bioagent identifying amplicons are obtained using the polymerase chain reaction (PCR) that is a routine method to those with ordinary skill in the molecular biology arts. Other amplification methods may be used such as ligase chain reaction (LCR), low-stringency single primer PCR, and multiple strand displacement amplification (MDA). These methods are also known to those with ordinary skill.

B. Primers and Primer Pairs

[293] In some embodiments, the primers are designed to bind to conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and yield amplification products which provide variability sufficient to distinguish each individual bioagent, and which are amenable to molecular mass analysis. In some embodiments, the highly conserved sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity, or between about 99-100% identity. The molecular mass of a given amplification product provides a means of identifying the bioagent from which it was obtained, due to the variability of the variable region. Thus, design of the primers involves selection of a variable region with sufficient variability to resolve the identity of a given bioagent. In some embodiments, bioagent identifying amplicons are specific to the identity of the bioagent.

[294] In some embodiments, identification of bioagents is accomplished at different levels using primers suited to resolution of each individual level of identification. Broad range survey primers are designed with the objective of identifying a bioagent as a member of a particular division (e.g., an order, family, genus or other such grouping of bioagents above the species level of bioagents). In some embodiments, broad range survey intelligent primers are capable of identification of bioagents at the species or sub-species level. Examples of broad range survey primers include, but are not limited to: primer pair numbers: 346 (SEQ ID NOs: 202:1110), 347 (SEQ ID NOs: 560:1278), 348 (SEQ ID NOs: 706:895), and 361 (SEQ ID NOs: 697:1398) which target DNA encoding 16S rRNA, and primer pair numbers 349 (SEQ ID NOs: 401:1156) and 360 (SEQ ID NOs: 409:1434) which target DNA encoding 23S rRNA.

[295] In some embodiments, drill-down primers are designed with the objective of identifying a bioagent at the sub-species level (including strains, subtypes, variants and isolates) based on sub-species characteristics which may, for example, include single nucleotide polymorphisms (SNPs), variable

number tandem repeats (VNTRs), deletions, drug resistance mutations or any other modification of a nucleic acid sequence of a bioagent relative to other members of a species having different sub-species characteristics. Drill-down intelligent primers are not always required for identification at the sub-species level because broad range survey intelligent primers may, in some cases provide sufficient identification resolution to accomplishing this identification objective. Examples of drill-down primers include, but are not limited to: confirmation primer pairs such as primer pair numbers 351 (SEQ ID NOs: 355:1423) and 353 (SEQ ID NOs: 220:1394), which target the pX01 virulence plasmid of *Bacillus anthracis*. Other examples of drill-down primer pairs are found in sets of triangulation genotyping primer pairs such as, for example, the primer pair number 2146 (SEQ ID NOs: 437:1137) which targets the *arcC* gene (encoding carbamate kinase) and is included in an 8 primer pair panel or kit for use in genotyping *Staphylococcus aureus*, or in other panels or kits of primer pairs used for determining drug-resistant bacterial strains, such as, for example, primer pair number 2095 (SEQ ID NOs: 456:1261) which targets the *pv-luk* gene (encoding Pantone-Valentine leukocidin) and is included in an 8 primer pair panel or kit for use in identification of drug resistant strains of *Staphylococcus aureus*.

[296] A representative process flow diagram used for primer selection and validation process is outlined in Figure 1. For each group of organisms, candidate target sequences are identified (200) from which nucleotide alignments are created (210) and analyzed (220). Primers are then designed by selecting appropriate priming regions (230) to facilitate the selection of candidate primer pairs (240). The primer pairs are then subjected to *in silico* analysis by electronic PCR (ePCR) (300) wherein bioagent identifying amplicons are obtained from sequence databases such as GenBank or other sequence collections (310) and checked for specificity *in silico* (320). Bioagent identifying amplicons obtained from GenBank sequences (310) can also be analyzed by a probability model which predicts the capability of a given amplicon to identify unknown bioagents such that the base compositions of amplicons with favorable probability scores are then stored in a base composition database (325). Alternatively, base compositions of the bioagent identifying amplicons obtained from the primers and GenBank sequences can be directly entered into the base composition database (330). Candidate primer pairs (240) are validated by testing their ability to hybridize to target nucleic acid by an *in vitro* amplification by a method such as PCR analysis (400) of nucleic acid from a collection of organisms (410). Amplification products thus obtained are analyzed by gel electrophoresis or by mass spectrometry to confirm the sensitivity, specificity and reproducibility of the primers used to obtain the amplification products (420).

[297] Many of the important pathogens, including the organisms of greatest concern as biowarfare agents, have been completely sequenced. This effort has greatly facilitated the design of primers for the detection of unknown bioagents. The combination of broad-range priming with division-wide and drill-down priming has been used very successfully in several applications of the technology, including

environmental surveillance for biowarfare threat agents and clinical sample analysis for medically important pathogens.

[298] Synthesis of primers is well known and routine in the art. The primers may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed.

[299] In some embodiments primers are employed as compositions for use in methods for identification of bacterial bioagents as follows: a primer pair composition is contacted with nucleic acid (such as, for example, bacterial DNA or DNA reverse transcribed from the rRNA) of an unknown bacterial bioagent. The nucleic acid is then amplified by a nucleic acid amplification technique, such as PCR for example, to obtain an amplification product that represents a bioagent identifying amplicon. The molecular mass of each strand of the double-stranded amplification product is determined by a molecular mass measurement technique such as mass spectrometry for example, wherein the two strands of the double-stranded amplification product are separated during the ionization process. In some embodiments, the mass spectrometry is electrospray Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) or electrospray time of flight mass spectrometry (ESI-TOF-MS). A list of possible base compositions can be generated for the molecular mass value obtained for each strand and the choice of the correct base composition from the list is facilitated by matching the base composition of one strand with a complementary base composition of the other strand. The molecular mass or base composition thus determined is then compared with a database of molecular masses or base compositions of analogous bioagent identifying amplicons for known viral bioagents. A match between the molecular mass or base composition of the amplification product and the molecular mass or base composition of an analogous bioagent identifying amplicon for a known viral bioagent indicates the identity of the unknown bioagent. In some embodiments, the primer pair used is one of the primer pairs of Table 2. In some embodiments, the method is repeated using one or more different primer pairs to resolve possible ambiguities in the identification process or to improve the confidence level for the identification assignment.

[300] In some embodiments, a bioagent identifying amplicon may be produced using only a single primer (either the forward or reverse primer of any given primer pair), provided an appropriate amplification method is chosen, such as, for example, low stringency single primer PCR (LSSP-PCR). Adaptation of this amplification method in order to produce bioagent identifying amplicons can be accomplished by one with ordinary skill in the art without undue experimentation.

[301] In some embodiments, the oligonucleotide primers are broad range survey primers which hybridize to conserved regions of nucleic acid encoding the hexon gene of all (or between 80% and 100%, between 85% and 100%, between 90% and 100% or between 95% and 100%) known bacteria and produce bacterial bioagent identifying amplicons.

[302] In some cases, the molecular mass or base composition of a bacterial bioagent identifying amplicon defined by a broad range survey primer pair does not provide enough resolution to unambiguously identify a bacterial bioagent at or below the species level. These cases benefit from further analysis of one or more bacterial bioagent identifying amplicons generated from at least one additional broad range survey primer pair or from at least one additional division-wide primer pair. The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as triangulation identification.

[303] In other embodiments, the oligonucleotide primers are division-wide primers which hybridize to nucleic acid encoding genes of species within a genus of bacteria. In other embodiments, the oligonucleotide primers are drill-down primers which enable the identification of sub-species characteristics. Drill down primers provide the functionality of producing bioagent identifying amplicons for drill-down analyses such as strain typing when contacted with nucleic acid under amplification conditions. Identification of such sub-species characteristics is often critical for determining proper clinical treatment of viral infections. In some embodiments, sub-species characteristics are identified using only broad range survey primers and division-wide and drill-down primers are not used.

[304] In some embodiments, the primers used for amplification hybridize to and amplify genomic DNA, and DNA of bacterial plasmids.

[305] In some embodiments, various computer software programs may be used to aid in design of primers for amplification reactions such as *Primer Premier 5* (Premier Biosoft, Palo Alto, CA) or *OLIGO* Primer Analysis Software (Molecular Biology Insights, Cascade, CO). These programs allow the user to input desired hybridization conditions such as melting temperature of a primer-template duplex for example. In some embodiments, an *in silico* PCR search algorithm, such as (ePCR) is used to analyze primer specificity across a plurality of template sequences which can be readily obtained from public sequence databases such as GenBank for example. An existing RNA structure search algorithm (Macke et al., Nucl. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (SantaLucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated herein by reference in its entirety). This also provides information on primer specificity of

the selected primer pairs. In some embodiments, the hybridization conditions applied to the algorithm can limit the results of primer specificity obtained from the algorithm. In some embodiments, the melting temperature threshold for the primer template duplex is specified to be 35°C or a higher temperature. In some embodiments the number of acceptable mismatches is specified to be seven mismatches or less. In some embodiments, the buffer components and concentrations and primer concentrations may be specified and incorporated into the algorithm, for example, an appropriate primer concentration is about 250 nM and appropriate buffer components are 50 mM sodium or potassium and 1.5 mM Mg²⁺.

[306] One with ordinary skill in the art of design of amplification primers will recognize that a given primer need not hybridize with 100% complementarity in order to effectively prime the synthesis of a complementary nucleic acid strand in an amplification reaction. Moreover, a primer may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event. (e.g., for example, a loop structure or a hairpin structure). The primers of the present invention may comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity with any of the primers listed in Table 2. Thus, in some embodiments of the present invention, an extent of variation of 70% to 100%, or any range therebetween, of the sequence identity is possible relative to the specific primer sequences disclosed herein. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is identical to another 20 nucleobase primer having two non-identical residues has 18 of 20 identical residues (18/20 = 0.9 or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of primer 20 nucleobases in length would have 15/20 = 0.75 or 75% sequence identity with the 20 nucleobase primer.

[307] Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison WI), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). In some embodiments, complementarity of primers with respect to the conserved priming regions of viral nucleic acid is between about 70% and about 75% 80%. In other embodiments, homology, sequence identity or complementarity, is between about 75% and about 80%. In yet other embodiments, homology, sequence identity or complementarity, is at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or is 100%.

[308] In some embodiments, the primers described herein comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%,

or at least 99%, or 100% (or any range therewithin) sequence identity with the primer sequences specifically disclosed herein.

[309] One with ordinary skill is able to calculate percent sequence identity or percent sequence homology and able to determine, without undue experimentation, the effects of variation of primer sequence identity on the function of the primer in its role in priming synthesis of a complementary strand of nucleic acid for production of an amplification product of a corresponding bioagent identifying amplicon.

[310] In one embodiment, the primers are at least 13 nucleobases in length. In another embodiment, the primers are less than 36 nucleobases in length.

[311] In some embodiments of the present invention, the oligonucleotide primers are 13 to 35 nucleobases in length (13 to 35 linked nucleotide residues). These embodiments comprise oligonucleotide primers 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 nucleobases in length, or any range therewithin. The present invention contemplates using both longer and shorter primers. Furthermore, the primers may also be linked to one or more other desired moieties, including, but not limited to, affinity groups, ligands, regions of nucleic acid that are not complementary to the nucleic acid to be amplified, labels, etc. Primers may also form hairpin structures. For example, hairpin primers may be used to amplify short target nucleic acid molecules. The presence of the hairpin may stabilize the amplification complex (see e.g., TAQMAN MicroRNA Assays, Applied Biosystems, Foster City, California).

[312] In some embodiments, any oligonucleotide primer pair may have one or both primers with less than 70% sequence homology with a corresponding member of any of the primer pairs of Table 2 if the primer pair has the capability of producing an amplification product corresponding to a bioagent identifying amplicon. In other embodiments, any oligonucleotide primer pair may have one or both primers with a length greater than 35 nucleobases if the primer pair has the capability of producing an amplification product corresponding to a bioagent identifying amplicon.

[313] In some embodiments, the function of a given primer may be substituted by a combination of two or more primers segments that hybridize adjacent to each other or that are linked by a nucleic acid loop structure or linker which allows a polymerase to extend the two or more primers in an amplification reaction.

[314] In some embodiments, the primer pairs used for obtaining bioagent identifying amplicons are the primer pairs of Table 2. In other embodiments, other combinations of primer pairs are possible by combining certain members of the forward primers with certain members of the reverse primers. An example can be seen in Table 2 for two primer pair combinations of forward primer 16S_EC_789_810_F (SEQ ID NO: 206), with the reverse primers 16S_EC_880_894_R (SEQ ID NO: 796), or 16S_EC_882_899_R or (SEQ ID NO: 818). Arriving at a favorable alternate combination of primers in a primer pair depends upon the properties of the primer pair, most notably the size of the bioagent identifying amplicon that would be produced by the primer pair, which preferably is between about 45 to about 150 nucleobases in length. Alternatively, a bioagent identifying amplicon longer than 150 nucleobases in length could be cleaved into smaller segments by cleavage reagents such as chemical reagents, or restriction enzymes, for example.

[315] In some embodiments, the primers are configured to amplify nucleic acid of a bioagent to produce amplification products that can be measured by mass spectrometry and from whose molecular masses candidate base compositions can be readily calculated.

[316] In some embodiments, any given primer comprises a modification comprising the addition of a non-templated T residue to the 5' end of the primer (i.e., the added T residue does not necessarily hybridize to the nucleic acid being amplified). The addition of a non-templated T residue has an effect of minimizing the addition of non-templated adenosine residues as a result of the non-specific enzyme activity of *Taq* polymerase (Magnuson et al., *Biotechniques*, 1996, 21, 700-709), an occurrence which may lead to ambiguous results arising from molecular mass analysis.

[317] In some embodiments of the present invention, primers may contain one or more universal bases. Because any variation (due to codon wobble in the 3rd position) in the conserved regions among species is likely to occur in the third position of a DNA (or RNA) triplet, oligonucleotide primers can be designed such that the nucleotide corresponding to this position is a base which can bind to more than one nucleotide, referred to herein as a "universal nucleobase." For example, under this "wobble" pairing, inosine (I) binds to U, C or A; guanine (G) binds to U or C, and uridine (U) binds to U or C. Other examples of universal nucleobases include nitroindoles such as 5-nitroindole or 3-nitropyrrrole (Loakes et al., *Nucleosides and Nucleotides*, 1995, 14, 1001-1003), the degenerate nucleotides dP or dK (Hill *et al.*), an acyclic nucleoside analog containing 5-nitroindazole (Van Aerschot et al., *Nucleosides and Nucleotides*, 1995, 14, 1053-1056) or the purine analog 1-(2-deoxy- β -D-ribofuranosyl)-imidazole-4-carboxamide (Sala et al., *Nucl. Acids Res.*, 1996, 24, 3302-3306).

[318] In some embodiments, to compensate for the somewhat weaker binding by the wobble base, the oligonucleotide primers are designed such that the first and second positions of each triplet are occupied by nucleotide analogs that bind with greater affinity than the unmodified nucleotide. Examples of these analogs include, but are not limited to, 2,6-diaminopurine which binds to thymine, 5-propynyluracil (also known as propynylated thymine) which binds to adenine and 5-propynylcytosine and phenoxazines, including G-clamp, which binds to G. Propynylated pyrimidines are described in U.S. Patent Nos. 5,645,985, 5,830,653 and 5,484,908, each of which is commonly owned and incorporated herein by reference in its entirety. Propynylated primers are described in U.S. Pre-Grant Publication No. 2003-0170682, which is also commonly owned and incorporated herein by reference in its entirety. Phenoxazines are described in U.S. Patent Nos. 5,502,177, 5,763,588, and 6,005,096, each of which is incorporated herein by reference in its entirety. G-clamps are described in U.S. Patent Nos. 6,007,992 and 6,028,183, each of which is incorporated herein by reference in its entirety.

[319] In some embodiments, primer hybridization is enhanced using primers containing 5-propynyl deoxy-cytidine and deoxy-thymidine nucleotides. These modified primers offer increased affinity and base pairing selectivity.

[320] In some embodiments, non-template primer tags are used to increase the melting temperature (T_m) of a primer-template duplex in order to improve amplification efficiency. A non-template tag is at least three consecutive A or T nucleotide residues on a primer which are not complementary to the template. In any given non-template tag, A can be replaced by C or G and T can also be replaced by C or G. Although Watson-Crick hybridization is not expected to occur for a non-template tag relative to the template, the extra hydrogen bond in a G-C pair relative to an A-T pair confers increased stability of the primer-template duplex and improves amplification efficiency for subsequent cycles of amplification when the primers hybridize to strands synthesized in previous cycles.

[321] In other embodiments, propynylated tags may be used in a manner similar to that of the non-template tag, wherein two or more 5-propynylcytidine or 5-propynyluridine residues replace template matching residues on a primer. In other embodiments, a primer contains a modified internucleoside linkage such as a phosphorothioate linkage, for example.

[322] In some embodiments, the primers contain mass-modifying tags. Reducing the total number of possible base compositions of a nucleic acid of specific molecular weight provides a means of avoiding a persistent source of ambiguity in determination of base composition of amplification products. Addition of mass-modifying tags to certain nucleobases of a given primer will result in simplification of *de novo* determination of base composition of a given bioagent identifying amplicon from its molecular mass.

[323] In some embodiments of the present invention, the mass modified nucleobase comprises one or more of the following: for example, 7-deaza-2'-deoxyadenosine-5'-triphosphate, 5-iodo-2'-deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxycytidine-5'-triphosphate, 5-iodo-2'-deoxycytidine-5'-triphosphate, 5-hydroxy-2'-deoxyuridine-5'-triphosphate, 4-thiothymidine-5'-triphosphate, 5-aza-2'-deoxyuridine-5'-triphosphate, 5-fluoro-2'-deoxyuridine-5'-triphosphate, O6-methyl-2'-deoxyguanosine-5'-triphosphate, N2-methyl-2'-deoxyguanosine-5'-triphosphate, 8-oxo-2'-deoxyguanosine-5'-triphosphate or thiothymidine-5'-triphosphate. In some embodiments, the mass-modified nucleobase comprises ^{15}N or ^{13}C or both ^{15}N and ^{13}C .

[324] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with a plurality of primer pairs. The advantages of multiplexing are that fewer reaction containers (for example, wells of a 96- or 384-well plate) are needed for each molecular mass measurement, providing time, resource and cost savings because additional bioagent identification data can be obtained within a single analysis. Multiplex amplification methods are well known to those with ordinary skill and can be developed without undue experimentation. However, in some embodiments, one useful and non-obvious step in selecting a plurality candidate bioagent identifying amplicons for multiplex amplification is to ensure that each strand of each amplification product will be sufficiently different in molecular mass that mass spectral signals will not overlap and lead to ambiguous analysis results. In some embodiments, a 10 Da difference in mass of two strands of one or more amplification products is sufficient to avoid overlap of mass spectral peaks.

[325] In some embodiments, as an alternative to multiplex amplification, single amplification reactions can be pooled before analysis by mass spectrometry. In these embodiments, as for multiplex amplification embodiments, it is useful to select a plurality of candidate bioagent identifying amplicons to ensure that each strand of each amplification product will be sufficiently different in molecular mass that mass spectral signals will not overlap and lead to ambiguous analysis results.

C Determination of Molecular Mass of Bioagent Identifying Amplicons

[326] In some embodiments, the molecular mass of a given bioagent identifying amplicon is determined by mass spectrometry. Mass spectrometry has several advantages, not the least of which is high bandwidth characterized by the ability to separate (and isolate) many molecular peaks across a broad range of mass to charge ratio (m/z). Thus mass spectrometry is intrinsically a parallel detection scheme without the need for radioactive or fluorescent labels, since every amplification product is identified by its molecular mass. The current state of the art in mass spectrometry is such that less than femtomole quantities of material can be readily analyzed to afford information about the molecular contents of the

sample. An accurate assessment of the molecular mass of the material can be quickly obtained, irrespective of whether the molecular weight of the sample is several hundred, or in excess of one hundred thousand atomic mass units (amu) or Daltons.

[327] In some embodiments, intact molecular ions are generated from amplification products using one of a variety of ionization techniques to convert the sample to gas phase. These ionization methods include, but are not limited to, electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI) and fast atom bombardment (FAB). Upon ionization, several peaks are observed from one sample due to the formation of ions with different charges. Averaging the multiple readings of molecular mass obtained from a single mass spectrum affords an estimate of molecular mass of the bioagent identifying amplicon. Electrospray ionization mass spectrometry (ESI-MS) is particularly useful for very high molecular weight polymers such as proteins and nucleic acids having molecular weights greater than 10 kDa, since it yields a distribution of multiply-charged molecules of the sample without causing a significant amount of fragmentation.

[328] The mass detectors used in the methods of the present invention include, but are not limited to, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), time of flight (TOF), ion trap, quadrupole, magnetic sector, Q-TOF, and triple quadrupole.

D. Base Compositions of Bioagent Identifying Amplicons

[329] Although the molecular mass of amplification products obtained using intelligent primers provides a means for identification of bioagents, conversion of molecular mass data to a base composition signature is useful for certain analyses. As used herein, "base composition" is the exact number of each nucleobase (A, T, C and G) determined from the molecular mass of a bioagent identifying amplicon. In some embodiments, a base composition provides an index of a specific organism. Base compositions can be calculated from known sequences of known bioagent identifying amplicons and can be experimentally determined by measuring the molecular mass of a given bioagent identifying amplicon, followed by determination of all possible base compositions which are consistent with the measured molecular mass within acceptable experimental error. The following example illustrates determination of base composition from an experimentally obtained molecular mass of a 46-mer amplification product originating at position 1337 of the 16S rRNA of *Bacillus anthracis*. The forward and reverse strands of the amplification product have measured molecular masses of 14208 and 14079 Da, respectively. The possible base compositions derived from the molecular masses of the forward and reverse strands for the *B. anthracis* products are listed in Table 1.

Table 1

Possible Base Compositions for *B. anthracis* 46mer Amplification Product

Calc. Mass Forward Strand	Mass Error Forward Strand	Base Composition of Forward Strand	Calc. Mass Reverse Strand	Mass Error Reverse Strand	Base Composition of Reverse Strand
14208.2935	0.079520	A1 G17 C10 T18	14079.2624	0.080600	A0 G14 C13 T19
14208.3160	0.056980	A1 G20 C15 T10	14079.2849	0.058060	A0 G17 C18 T11
14208.3386	0.034440	A1 G23 C20 T2	14079.3075	0.035520	A0 G20 C23 T3
14208.3074	0.065560	A6 G11 C3 T26	14079.2538	0.089180	A5 G5 C1 T35
14208.3300	0.043020	A6 G14 C8 T18	14079.2764	0.066640	A5 G8 C5 T27
14208.3525	0.020480	A6 G17 C13 T10	14079.2989	0.044100	A5 G11 C11 T19
14208.3751	0.002060	A6 G20 C18 T2	14079.3214	0.021560	A5 G14 C16 T11
14208.3439	0.029060	A11 G8 C1 T26	14079.3440	0.000980	A5 G17 C21 T3
14208.3665	0.006520	A11 G11 C6 T18	14079.3129	0.030140	A10 G5 C4 T27
14208.3890	0.016020	A11 G14 C11 T10	14079.3354	0.007600	A10 G8 C9 T19
14208.4116	0.038560	A11 G17 C16 T2	14079.3579	0.014940	A10 G11 C14 T11
14208.4030	0.029980	A16 G8 C4 T18	14079.3805	0.037480	A10 G14 C19 T3
14208.4255	0.052520	A16 G11 C9 T10	14079.3494	0.006360	A15 G2 C2 T27
14208.4481	0.075060	A16 G14 C14 T2	14079.3719	0.028900	A15 G5 C7 T19
14208.4395	0.066480	A21 G5 C2 T18	14079.3944	0.051440	A15 G8 C12 T3
14208.4620	0.089020	A21 G8 C7 T10	14079.4170	0.073980	A15 G11 C17 T3
-	-	-	14079.4084	0.065400	A20 G2 C5 T19
-	-	-	14079.4309	0.087940	A20 G5 C10 T13

[330] Among the 16 possible base compositions for the forward strand and the 18 possible base compositions for the reverse strand that were calculated, only one pair (shown in **bold**) are complementary base compositions, which indicates the true base composition of the amplification product. It should be recognized that this logic is applicable for determination of base compositions of any bioagent identifying amplicon, regardless of the class of bioagent from which the corresponding amplification product was obtained.

[331] In some embodiments, assignment of previously unobserved base compositions (also known as "true unknown base compositions") to a given phylogeny can be accomplished via the use of pattern classifier model algorithms. Base compositions, like sequences, vary slightly from strain to strain within species, for example. In some embodiments, the pattern classifier model is the mutational probability model. On other embodiments, the pattern classifier is the polytope model. The mutational probability model and polytope model are both commonly owned and described in U.S. Patent application Serial No. 11/073,362 which is incorporated herein by reference in entirety.

[332] In one embodiment, it is possible to manage this diversity by building "base composition probability clouds" around the composition constraints for each species. This permits identification of

organisms in a fashion similar to sequence analysis. A “pseudo four-dimensional plot” can be used to visualize the concept of base composition probability clouds. Optimal primer design requires optimal choice of bioagent identifying amplicons and maximizes the separation between the base composition signatures of individual bioagents. Areas where clouds overlap indicate regions that may result in a misclassification, a problem which is overcome by a triangulation identification process using bioagent identifying amplicons not affected by overlap of base composition probability clouds.

[333] In some embodiments, base composition probability clouds provide the means for screening potential primer pairs in order to avoid potential misclassifications of base compositions. In other embodiments, base composition probability clouds provide the means for predicting the identity of a bioagent whose assigned base composition was not previously observed and/or indexed in a bioagent identifying amplicon base composition database due to evolutionary transitions in its nucleic acid sequence. Thus, in contrast to probe-based techniques, mass spectrometry determination of base composition does not require prior knowledge of the composition or sequence in order to make the measurement.

[334] The present invention provides bioagent classifying information similar to DNA sequencing and phylogenetic analysis at a level sufficient to identify a given bioagent. Furthermore, the process of determination of a previously unknown base composition for a given bioagent (for example, in a case where sequence information is unavailable) has downstream utility by providing additional bioagent indexing information with which to populate base composition databases. The process of future bioagent identification is thus greatly improved as more BCS indexes become available in base composition databases.

E. Triangulation Identification

[335] In some cases, a molecular mass of a single bioagent identifying amplicon alone does not provide enough resolution to unambiguously identify a given bioagent. The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as “triangulation identification.” Triangulation identification is pursued by determining the molecular masses of a plurality of bioagent identifying amplicons selected within a plurality of housekeeping genes. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical of *B. anthracis* (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the *B. anthracis* genome would suggest a genetic engineering event.

[336] In some embodiments, the triangulation identification process can be pursued by characterization of bioagent identifying amplicons in a massively parallel fashion using the polymerase chain reaction (PCR), such as multiplex PCR where multiple primers are employed in the same amplification reaction mixture, or PCR in multi-well plate format wherein a different and unique pair of primers is used in multiple wells containing otherwise identical reaction mixtures. Such multiplex and multi-well PCR methods are well known to those with ordinary skill in the arts of rapid throughput amplification of nucleic acids. In other related embodiments, one PCR reaction per well or container may be carried out, followed by an amplicon pooling step wherein the amplification products of different wells are combined in a single well or container which is then subjected to molecular mass analysis. The combination of pooled amplicons can be chosen such that the expected ranges of molecular masses of individual amplicons are not overlapping and thus will not complicate identification of signals.

F. Codon Base Composition Analysis

[337] In some embodiments of the present invention, one or more nucleotide substitutions within a codon of a gene of an infectious organism confer drug resistance upon an organism which can be determined by codon base composition analysis. The organism can be a bacterium, virus, fungus or protozoan.

[338] In some embodiments, the amplification product containing the codon being analyzed is of a length of about 35 to about 200 nucleobases. The primers employed in obtaining the amplification product can hybridize to upstream and downstream sequences directly adjacent to the codon, or can hybridize to upstream and downstream sequences one or more sequence positions away from the codon. The primers may have between about 70% to 100% sequence complementarity with the sequence of the gene containing the codon being analyzed.

[339] In some embodiments, the codon base composition analysis is undertaken

[340] In some embodiments, the codon analysis is undertaken for the purpose of investigating genetic disease in an individual. In other embodiments, the codon analysis is undertaken for the purpose of investigating a drug resistance mutation or any other deleterious mutation in an infectious organism such as a bacterium, virus, fungus or protozoan. In some embodiments, the bioagent is a bacterium identified in a biological product.

[341] In some embodiments, the molecular mass of an amplification product containing the codon being analyzed is measured by mass spectrometry. The mass spectrometry can be either electrospray (ESI) mass spectrometry or matrix-assisted laser desorption ionization (MALDI) mass spectrometry.

Time-of-flight (TOF) is an example of one mode of mass spectrometry compatible with the analyses of the present invention.

[342] The methods of the present invention can also be employed to determine the relative abundance of drug resistant strains of the organism being analyzed. Relative abundances can be calculated from amplitudes of mass spectral signals with relation to internal calibrants. In some embodiments, known quantities of internal amplification calibrants can be included in the amplification reactions and abundances of analyte amplification product estimated in relation to the known quantities of the calibrants.

[343] In some embodiments, upon identification of one or more drug-resistant strains of an infectious organism infecting an individual, one or more alternative treatments can be devised to treat the individual.

G. Determination of the Quantity of a Bioagent

[344] In some embodiments, the identity and quantity of an unknown bioagent can be determined using the process illustrated in Figure 2. Primers (500) and a known quantity of a calibration polynucleotide (505) are added to a sample containing nucleic acid of an unknown bioagent. The total nucleic acid in the sample is then subjected to an amplification reaction (510) to obtain amplification products. The molecular masses of amplification products are determined (515) from which are obtained molecular mass and abundance data. The molecular mass of the bioagent identifying amplicon (520) provides the means for its identification (525) and the molecular mass of the calibration amplicon obtained from the calibration polynucleotide (530) provides the means for its identification (535). The abundance data of the bioagent identifying amplicon is recorded (540) and the abundance data for the calibration data is recorded (545), both of which are used in a calculation (550) which determines the quantity of unknown bioagent in the sample.

[345] A sample comprising an unknown bioagent is contacted with a pair of primers that provide the means for amplification of nucleic acid from the bioagent, and a known quantity of a polynucleotide that comprises a calibration sequence. The nucleic acids of the bioagent and of the calibration sequence are amplified and the rate of amplification is reasonably assumed to be similar for the nucleic acid of the bioagent and of the calibration sequence. The amplification reaction then produces two amplification products: a bioagent identifying amplicon and a calibration amplicon. The bioagent identifying amplicon and the calibration amplicon should be distinguishable by molecular mass while being amplified at essentially the same rate. Effecting differential molecular masses can be accomplished by choosing as a calibration sequence, a representative bioagent identifying amplicon (from a specific species of bioagent) and performing, for example, a 2-8 nucleobase deletion or insertion within the variable region between

the two priming sites. The amplified sample containing the bioagent identifying amplicon and the calibration amplicon is then subjected to molecular mass analysis by mass spectrometry, for example. The resulting molecular mass analysis of the nucleic acid of the bioagent and of the calibration sequence provides molecular mass data and abundance data for the nucleic acid of the bioagent and of the calibration sequence. The molecular mass data obtained for the nucleic acid of the bioagent enables identification of the unknown bioagent and the abundance data enables calculation of the quantity of the bioagent, based on the knowledge of the quantity of calibration polynucleotide contacted with the sample.

[346] In some embodiments, construction of a standard curve where the amount of calibration polynucleotide spiked into the sample is varied provides additional resolution and improved confidence for the determination of the quantity of bioagent in the sample. The use of standard curves for analytical determination of molecular quantities is well known to one with ordinary skill and can be performed without undue experimentation.

[347] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with multiple primer pairs which also amplify the corresponding standard calibration sequences. In this or other embodiments, the standard calibration sequences are optionally included within a single vector which functions as the calibration polynucleotide. Multiplex amplification methods are well known to those with ordinary skill and can be performed without undue experimentation.

[348] In some embodiments, the calibrant polynucleotide is used as an internal positive control to confirm that amplification conditions and subsequent analysis steps are successful in producing a measurable amplicon. Even in the absence of copies of the genome of a bioagent, the calibration polynucleotide should give rise to a calibration amplicon. Failure to produce a measurable calibration amplicon indicates a failure of amplification or subsequent analysis step such as amplicon purification or molecular mass determination. Reaching a conclusion that such failures have occurred is in itself, a useful event.

[349] In some embodiments, the calibration sequence is comprised of DNA. In some embodiments, the calibration sequence is comprised of RNA.

[350] In some embodiments, the calibration sequence is inserted into a vector that itself functions as the calibration polynucleotide. In some embodiments, more than one calibration sequence is inserted into the vector that functions as the calibration polynucleotide. Such a calibration polynucleotide is herein termed a "combination calibration polynucleotide." The process of inserting polynucleotides into vectors

is routine to those skilled in the art and can be accomplished without undue experimentation. Thus, it should be recognized that the calibration method should not be limited to the embodiments described herein. The calibration method can be applied for determination of the quantity of any bioagent identifying amplicon when an appropriate standard calibrant polynucleotide sequence is designed and used. The process of choosing an appropriate vector for insertion of a calibrant is also a routine operation that can be accomplished by one with ordinary skill without undue experimentation.

H. Identification of Bacteria

[351] In other embodiments of the present invention, the primer pairs produce bioagent identifying amplicons within stable and highly conserved regions of bacteria. The advantage to characterization of an amplicon defined by priming regions that fall within a highly conserved region is that there is a low probability that the region will evolve past the point of primer recognition, in which case, the primer hybridization of the amplification step would fail. Such a primer set is thus useful as a broad range survey-type primer. In another embodiment of the present invention, the intelligent primers produce bioagent identifying amplicons including a region which evolves more quickly than the stable region described above. The advantage of characterization bioagent identifying amplicon corresponding to an evolving genomic region is that it is useful for distinguishing emerging strain variants or the presence of virulence genes, drug resistance genes, or codon mutations that induce drug resistance.

[352] The present invention also has significant advantages as a platform for identification of diseases caused by emerging bacterial strains such as, for example, drug-resistant strains of *Staphylococcus aureus*. The present invention eliminates the need for prior knowledge of bioagent sequence to generate hybridization probes. This is possible because the methods are not confounded by naturally occurring evolutionary variations occurring in the sequence acting as the template for production of the bioagent identifying amplicon. Measurement of molecular mass and determination of base composition is accomplished in an unbiased manner without sequence prejudice.

[353] Another embodiment of the present invention also provides a means of tracking the spread of a bacterium, such as a particular drug-resistant strain when a plurality of samples obtained from different locations are analyzed by the methods described above in an epidemiological setting. In one embodiment, a plurality of samples from a plurality of different locations is analyzed with primer pairs which produce bioagent identifying amplicons, a subset of which contains a specific drug-resistant bacterial strain. The corresponding locations of the members of the drug-resistant strain subset indicate the spread of the specific drug-resistant strain to the corresponding locations.

I. Kits

[354] The present invention also provides kits for carrying out the methods described herein. In some embodiments, the kit may comprise a sufficient quantity of one or more primer pairs to perform an amplification reaction on a target polynucleotide from a bioagent to form a bioagent identifying amplicon. In some embodiments, the kit may comprise from one to fifty primer pairs, from one to twenty primer pairs, from one to ten primer pairs, or from two to five primer pairs. In some embodiments, the kit may comprise one or more primer pairs recited in Table 2.

[355] In some embodiments, the kit comprises one or more broad range survey primer(s), division wide primer(s), or drill-down primer(s), or any combination thereof. If a given problem involves identification of a specific bioagent, the solution to the problem may require the selection of a particular combination of primers to provide the solution to the problem. A kit may be designed so as to comprise particular primer pairs for identification of a particular bioagent. A drill-down kit may be used, for example, to distinguish different genotypes or strains, drug-resistant, or otherwise. In some embodiments, the primer pair components of any of these kits may be additionally combined to comprise additional combinations of broad range survey primers and division-wide primers so as to be able to identify a bacterium.

[356] In some embodiments, the kit contains standardized calibration polynucleotides for use as internal amplification calibrants. Internal calibrants are described in commonly owned U.S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[357] In some embodiments, the kit comprises a sufficient quantity of reverse transcriptase (if RNA is to be analyzed for example), a DNA polymerase, suitable nucleoside triphosphates (including alternative dNTPs such as inosine or modified dNTPs such as the 5-propynyl pyrimidines or any dNTP containing molecular mass-modifying tags such as those described above), a DNA ligase, and/or reaction buffer, or any combination thereof, for the amplification processes described above. A kit may further include instructions pertinent for the particular embodiment of the kit, such instructions describing the primer pairs and amplification conditions for operation of the method. A kit may also comprise amplification reaction containers such as microcentrifuge tubes and the like. A kit may also comprise reagents or other materials for isolating bioagent nucleic acid or bioagent identifying amplicons from amplification, including, for example, detergents, solvents, or ion exchange resins which may be linked to magnetic beads. A kit may also comprise a table of measured or calculated molecular masses and/or base compositions of bioagents using the primer pairs of the kit.

[358] Some embodiments are kits that contain one or more survey bacterial primer pairs represented by primer pair compositions wherein each member of each pair of primers has 70% to 100% sequence

identity with the corresponding member from the group of primer pairs represented by any of the primer pairs of Table 5. The survey primer pairs may include broad range primer pairs which hybridize to ribosomal RNA, and may also include division-wide primer pairs which hybridize to housekeeping genes such as rplB, tufB, rpoB, rpoC, valS, and infB, for example.

[359] In some embodiments, a kit may contain one or more survey bacterial primer pairs and one or more triangulation genotyping analysis primer pairs such as the primer pairs of Tables 8, 12, 14, 19, 21, 23, or 24. In some embodiments, the kit may represent a less expansive genotyping analysis but include triangulation genotyping analysis primer pairs for more than one genus or species of bacteria. For example, a kit for surveying nosocomial infections at a health care facility may include, for example, one or more broad range survey primer pairs, one or more division wide primer pairs, one or more *Acinetobacter baumannii* triangulation genotyping analysis primer pairs and one or more *Staphylococcus aureus* triangulation genotyping analysis primer pairs. One with ordinary skill will be capable of analyzing *in silico* amplification data to determine which primer pairs will be able to provide optimal identification resolution for the bacterial bioagents of interest.

[360] In some embodiments, a kit may be assembled for identification of strains of bacteria involved in contamination of food. An example of such a kit embodiment is a kit comprising one or more bacterial survey primer pairs of Table 5 with one or more triangulation genotyping analysis primer pairs of Table 12 which provide strain resolving capabilities for identification of specific strains of *Campylobacter jejuni*.

[361] Some embodiments of the kits are 96-well or 384-well plates with a plurality of wells containing any or all of the following components: dNTPs, buffer salts, Mg^{2+} , betaine, and primer pairs. In some embodiments, a polymerase is also included in the plurality of wells of the 96-well or 384-well plates.

[362] Some embodiments of the kit contain instructions for PCR and mass spectrometry analysis of amplification products obtained using the primer pairs of the kits.

[363] Some embodiments of the kit include a barcode which uniquely identifies the kit and the components contained therein according to production lots and may also include any other information relative to the components such as concentrations, storage temperatures, etc. The barcode may also include analysis information to be read by optical barcode readers and sent to a computer controlling amplification, purification and mass spectrometric measurements. In some embodiments, the barcode provides access to a subset of base compositions in a base composition database which is in digital

communication with base composition analysis software such that a base composition measured with primer pairs from a given kit can be compared with known base compositions of bioagent identifying amplicons defined by the primer pairs of that kit.

[364] In some embodiments, the kit contains a database of base compositions of bioagent identifying amplicons defined by the primer pairs of the kit. The database is stored on a convenient computer readable medium such as a compact disk or USB drive, for example.

[365] In some embodiments, the kit includes a computer program stored on a computer formatted medium (such as a compact disk or portable USB disk drive, for example) comprising instructions which direct a processor to analyze data obtained from the use of the primer pairs of the present invention. The instructions of the software transform data related to amplification products into a molecular mass or base composition which is a useful concrete and tangible result used in identification and/or classification of bioagents. In some embodiments, the kits of the present invention contain all of the reagents sufficient to carry out one or more of the methods described herein.

[366] While the present invention has been described with specificity in accordance with certain of its embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same. In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner.

EXAMPLES

Example 1: Design and Validation of Primers that Define Bioagent Identifying Amplicons for Identification of Bacteria

[367] For design of primers that define bacterial bioagent identifying amplicons, a series of bacterial genome segment sequences were obtained, aligned and scanned for regions where pairs of PCR primers would amplify products of about 45 to about 150 nucleotides in length and distinguish subgroups and/or individual strains from each other by their molecular masses or base compositions. A typical process shown in Figure 1 is employed for this type of analysis.

[368] A database of expected base compositions for each primer region was generated using an *in silico* PCR search algorithm, such as (ePCR). An existing RNA structure search algorithm (Macke et al., Nucl. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (Santa Lucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated

herein by reference in its entirety). This also provides information on primer specificity of the selected primer pairs.

[369] Table 2 represents a collection of primers (sorted by primer pair number) designed to identify bacteria using the methods described herein. The primer pair number is an in-house database index number. Primer sites were identified on segments of genes, such as, for example, the 16S rRNA gene. The forward or reverse primer name shown in Table 2 indicates the gene region of the bacterial genome to which the primer hybridizes relative to a reference sequence. In Table 2, for example, the forward primer name 16S_EC_1077_1106_F indicates that the forward primer (F) hybridizes to residues 1077-1106 of the reference sequence represented by a sequence extraction of coordinates 4033120..4034661 from GenBank gi number 16127994 (as indicated in Table 3). As an additional example: the forward primer name BONTA_X52066_450_473 indicates that the primer hybridizes to residues 450-437 of the gene encoding *Clostridium botulinum* neurotoxin type A (BoNT/A) represented by GenBank Accession No. X52066 (primer pair name codes appearing in Table 2 are defined in Table 3. One with ordinary skill knows how to obtain individual gene sequences or portions thereof from genomic sequences present in GenBank. In Table 2, Tp = 5-propynyluracil; Cp = 5-propynylcytosine; * = phosphorothioate linkage; I = inosine. T. GenBank Accession Numbers for reference sequences of bacteria are shown in Table 3 (below). In some cases, the reference sequences are extractions from bacterial genomic sequences or complements thereof.

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Table 2: Primer Pairs for Identification of Bacteria

Primer Pair Number	Forward Primer Name	Forward Sequence	Forward SEQ ID NO.	Reverse Primer Name	Reverse Sequence	Reverse SEQ ID NO.
1	16S BC 1077 1106 F	GTGATGTGTGGGTTAATGATCCCGTAA	134	16S BC 1175 1195 R	GAATCATCTCCACACTCTCTC	809
2	16S BC 1082 1106 F	ATGTGGGTATGTCCTCCGACAGAG	38	16S BC 1175 1197 R	TTCAGTATCTCCACACTCTCTC	1398
3	16S BC 1090 1111 F	TTAATCTCCGACACATATCCGAA	651	16S BC 1175 1196 R	TGAAGTCTCCACACTCTCTC	1159
4	16S BC 1222 1241 F	GCTACACAGCTGTGCTATATG	114	16S BC 1303 1323 R	CGAGTTGACAGCTGATACCG	787
5	16S BC 1332 1353 F	ATGTCGAGATCTGTATATATG	10	16S BC 1389 1407 R	GACGGCGGTGTGTATGAG	806
6	16S BC 30 54 F	TGAGACCTGTGGGCTGCTTAAAC	429	16S BC 105 126 R	TTACTGACACCTCCGCGCT	897
7	16S BC 38 64 F	GTGCTATGCTCTATACATGCAATGTC	136	16S BC 101 120 R	TTACTGACACCTCCGCGCT	1365
8	16S BC 43 68 F	TACACATGCAATGCAATGTC	152	16S BC 104 120 R	TTACTGACACCTCCGCGCT	1364
9	16S BC 683 700 F	GTTGACGCGTGAATGTC	137	16S BC 774 795 R	GTATCTAATCTGTTTGTCTCC	839
10	16S BC 713 732 F	AGACACCGATGCGAAGGC	21	16S BC 789 809 R	CTGTGACATGCGGGTATCTA	798
11	16S BC 785 806 F	GGATTAGACATCTGTGTATCTC	118	16S BC 880 897 R	GGCGTACTCTCCGACGCG	830
12	16S BC 785 810 F	GGATTAGACATCTGTGTATCTC	119	16S BC 880 897 2 R	GGCGTACTCTCCGACGCG	830
13	16S BC 789 810 F	TGATATCTCTGTGTATCTC	206	16S BC 880 894 R	GTATCTCTCCGACGCG	796
14	16S BC 960 981 F	TTGATATGCAAGGAGAGAGACT	672	16S BC 1054 1073 R	ACGAGCTCCGACGCGCGT	735
15	16S BC 969 985 F	ACGAGAGACCTTAC	19	16S BC 1061 1078 R	ACGACAGAGCTGACGAC	734
16	23S BC 1926 1843 P	CTGACACTCTCCGCGTGC	80	23S BC 1906 1924 R	ACGCTGTATGATGACGCG	805
17	23S BC 2645 2669 F	TGTGCTCTCTGTATGACAGAGACCG	408	23S BC 2744 2761 R	TGCTTATGATGCTTACG	1252
18	23S BC 2645 2669 2_	CTGTCTCTCTGTATGACAGAGACCG	83	23S BC 2751 2767 R	GTTCATGCTGATGATCTTACG	846
19	23S BC 493 518 F	GGGAGTGTGAAGAGATCTGACGCG	125	23S BC 551 571 R	ACGAGAGTACGCTGACG	717
20	23S BC 493 518 2 F	GGGAGTGTGAAGAGATCTGACGCG	125	23S BC 551 571 2 R	ACGAGAGTACGCTGACG	716
21	23S BC 971 992 F	CGAGAGGAGGAGGAGGAGGAGGAGG	66	23S BC 1059 1077 R	TGCTCTCTGTATGACGAC	1282
22	CAPC BA 104 131 F	GTATTTATGACACTGCTTTTATATG	139	CAPC BA 180 205 R	TGATCTGTGAAGACGATGACG	1150
23	CAPC BA 114 132 F	ACTGTGTTTATATGACGCG	20	CAPC BA 185 205 R	TGATCTGTGAAGACGACG	1149
24	CAPC BA 274 301 F	GATTTATGTTATCTGATGATGACGAT	109	CAPC BA 349 376 R	GATGCTGTCTGATGATGATGACG	837
25	CAPC BA 276 296 F	TATTTATGTTATCTGATGATGACG	663	CAPC BA 356 377 R	GATGCTGTCTGATGATGATGACG	844
26	CAPC BA 281 301 F	GTATTTATGTTATGATGATGACG	138	CAPC BA 361 378 R	TGATGCTGTCTGATGATGATGACG	1288
27	CAPC BA 315 334 F	CGATGATGATGATGATGATGATG	59	CAPC BA 361 378 R	TGATGCTGTCTGATGATGATGACG	1288
28	CYA BA 1055 1072 F	GAAAGGATTCGATGATGATG	92	CYA BA 1112 1130 R	TGTTGACATGCTGCTCTGATG	1352

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29	CYA BA 1349 1370 F	ACACQAGCTACATCATCAGC	12	CYA BA 1447 1426 R	CTTCTACATTTTACCATAC	800
30	CYA BA 1355 1379 F	CGAATCTACATCAGACAGAGAG	64	CYA BA 1448 1467 R	TGTTAAGGCTTACAGACC	1342
31	CYA BA 1359 1379 F	ACATATCAGACAGACAGAGAG	13	CYA BA 1447 1467 R	CGGCTTTACAGACC	794
32	CYA BA 914 937 F	CAAGTTTATCTACAGACAGAGAG	53	CYA BA 999 1026 R	ACACTTTTAAAGTTTGTACACAC	728
33	CYA BA 916 935 F	GGTTATCTACAGACAGAGAG	131	CYA BA 1003 1025 R	CAATTTTATAGTTTGTAC	768
34	INF B EC 1365 1393 F	TGCTCGTGCTCAGATACAGAT	524	INF B EC 1439 1467 R	TGCTGCTTGCATGTTTATCTTCA	1248
35	LEF BA 1033 1052 F	TTA	254	LEF BA 1119 1135 R	A	803
36	LEF BA 1036 1066 F	CAAGACAGAGAGAGAGCTCTTAAAG	44	LEF BA 1119 1149 R	AGATTAAGATCAGATACATCATTTGT	745
37	LEF BA 756 781 F	AGCTTTGCTATTTATCAGAC	26	LEF BA 843 872 R	TGTTCCAGAGATAGATTTATTTCTGTT	1135
38	LEF BA 759 778 F	CTTTTGCAATTTATTCAGAC	90	LEF BA 843 865 R	AGATAGATTTATTTCTGTTG	748
39	LEF BA 755 813 F	TTTACAGCTTATGACAG	700	LEF BA 883 900 R	TCTGACAGATCGGTTG	1140
40	LEF BA 843 859 F	CAAGGATCTGACAG	43	LEF BA 939 958 R	CAATTAAGATCTGCTCAG	762
41	PAG BA 122 142 F	CAATATCAGTCTCCAGG	49	PAG BA 190 209 R	CTGTATGATAGAGGATAC	781
42	PAG BA 123 145 F	AGATCTAGTTTCCAGGCTTAC	22	PAG BA 387 210 R	CCGTATGATAGAGGATGACAG	774
43	PAG BA 369 287 F	AACTGCTATTTGCTGAG	11	PAG BA 325 344 R	TGATATCAGCGAGTAC	1186
44	PAG BA 655 675 F	GAAGATATCAGTCTATGATGTC	93	PAG BA 755 772 R	CGTGTCTGCTTTTAC	778
45	PAG BA 753 772 F	TCCTGAGAAATGAGACAG	341	PAG BA 849 868 R	TGATATGATCTCCAGAG	1089
46	PAG BA 763 781 F	TGAGACAGCTCTGATG	552	PAG BA 849 868 R	TGATATGATCTCCAGAG	1089
47	REOC EC 1018 1045 F	CAAACTATATGATAGCTGATGA	39	REOC EC 1095 1124 R	TCAGAGGCTATTTTGTGTAACAC	959
48	REOC EC 1018 1045 2 F	CT	39	REOC EC 1095 1124 2 R	AT	958
49	REOC EC 114 140 F	TAGAGCTGAGAGACATCAACTAC	158	REOC EC 213 232 R	GGGCTTGTACTTACGAC	831
50	REOC EC 2178 2196 F	TGATCTGTGCTCGTGTG	478	REOC EC 2225 2246 R	TGCTGCTACAGGCTGATAC	1414
51	REOC EC 2178 2196 2 F	TGATCTGTGCTCGTGTG	477	REOC EC 2225 2246 2 R	TGCTGCTACAGGCTGATAC	1413
52	REOC EC 2218 2241 F	CTGCGATGATGCTGCTGTGATG	81	REOC EC 2313 2337 R	CGACGCTGCTGCTGATGATGATG	790
53	REOC EC 2218 2241 2 F	CTGCTGTATGCTGCTGTGATG	86	REOC EC 2313 2337 2 R	CGACGCTGCTGCTGATGATGATG	789
54	REOC EC 808 833 F	CGTGGGATATACCTGATACAGCG	75	REOC EC 865 889 R	GTCTTCTGCTGCTGATGATGATG	847
55	REOC EC 808 833 2 F	CGTGGGATATACCTGATACAGCG	76	REOC EC 865 891 R	ACCTTTTCTGCTGCTGATGATGATG	741
56	REOC EC 993 1019 F	CAAGGATAGAGAGCTGTTTCTGTC	41	REOC EC 1036 1059 R	CAAGGCTGATGATGATGATGATG	785
57	REOC EC 993 1019 2 F	A	40	REOC EC 1036 1059 2 R	CAAGGCTGATGATGATGATGATG	784

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58	SPPE BA 115 137 F	CAAGCTAAGCCGACATCAAGAC	45	SPPE BA 137 222 R	TCCAGCTGCTGTTCACTTGCAGATTC	1201
59	TUPE EC 239 259 F	TGAGCTGCGAGAGACAGAGCTG	204	TUPE EC 283 303 R	GCCCTCCATCTGAGCAGACAC	815
60	TUPE EC 239 259 2 F	TGAGCTGCGAGAGACAGAGCTG	678	TUPE EC 303 303 2 R	GCCCTCCATCTGAGCAGACAC	816
61	TUPE EC 976 1000 F	AACTACAGCTCGCAGCTCTACTTCC	4	TUPE EC 1045 1068 R	GTTTGCCCGGACGACACCTTTC	845
62	TUPE EC 976 1000 2 F	AACTACAGCTCGCAGCTCTACTTCC	5	TUPE EC 1045 1068 2 R	GTTTGCCCGGACATTCACATCTTC	844
63	TUPE EC 985 1012 F	CCAGCTCTCTCTCTCTCTACACGCA	56	TUPE EC 1033 1062 R	TCCAGGCACTCACTTCTACTCTCTCT	1006
64	TUPE EC 985 1012 F	CCAGCTCTCTCTCTCTCTACACGCA	56	TUPE EC 1033 1062 R	TCCAGGCACTCTCTCTCTCTCTCTCT	1006
65	REPE EC 650 679 F	GACCTACGTAAGAGGTTCTCTAATG	98	REPE EC 739 762 R	TCCAGGCTGCTGTTTACCCCATG	999
66	REPE EC 688 710 F	CACTCACAAGTGCTGCTGTAAGG	54	REPE EC 735 757 R	GCTGCTGTTTACCCCATGAGT	842
67	REPE EC 688 710 F	CACTCACAAGTGCTGCTGTAAGG	54	REPE EC 735 757 R	ATTCCAGAGCCATCTCTTTGCTAAAC	842
68	RPOC EC 1036 1060 F	CGTGTGACATTCGCGGCGTTGAG	78	RPOC EC 1097 1126 R	TTCCTTGAAGGTATGAGCTGCTCGTA	754
69	RPOB EC 3782 3790 F	AGT	248	RPOB EC 3836 3865 R	TTCCTTGAAGGTATGAGCTGCTCGTA	1435
70	REPE EC 688 710 F	CACTCACAAGTGCTGCTGTAAGG	54	REPE EC 743 771 R	TGTTTGTATCTCAGAGTCTGTTTACCC	1356
71	VAUS EC 1105 1124 F	CGTGGCGCTGCTGTTGTA	77	VAUS EC 1195 1218 R	CGTTCGACATCGAGATCTGCGCTT	795
72	RPOB EC 1845 1865 F	TATGCTGCGAGACCTCGAC	233	RPOB EC 1909 1939 R	GCTCGATTCGCTTTCCTACG	825
73	REPE EC 669 698 F	TGATGAGACCTTATACATCTCCAC	623	REPE EC 735 761 R	CGATGCTGCTGTTTACCCATGAGTA	767
74	REPE EC 671 700 F	AGT	169	REPE EC 737 762 R	TCCAGGCTGCTGTTTACCCATGAG	1000
75	SP101 SP2T11 1 29 F	AACTCTTATGTAAGACCAACGAGA	2	SP101 SP2T11 92 116 R	CCCTACCCAGCTCTCCACGAGGCG	779
76	SP101 SP2T11 118 14 F	GCTGCGAAATTAACCAAGATGCT	115	SP101 SP2T11 213 238 R	TGTCGCGAGATCTCCACGCTGCTCT	1340
77	SP101 SP2T11 216 24 F	ACGCGTGTGTAATCCGACATGTA	24	SP101 SP2T11 308 333 R	TGCACTGTTGACAACTCTCTGTTGCTG	1209
78	SP101 SP2T11 266 29 F	CTGTACTTGTGCTGCAACGCGCT	89	SP101 SP2T11 355 380 R	GCTGCTTGTAGTGCTGATCCCTTC	824
79	SP101 SP2T11 312 34 F	GTCAATGTGCACTGTTTACGCT	132	SP101 SP2T11 423 441 R	ATCCCTGCTCTTCTCTGCTG	753
80	SP101 SP2T11 358 38 F	TCGATTCGACCACTGACACTAT	126	SP101 SP2T11 448 473 R	CGAATCTTTCACCACTGATCAGC	766
81	SP101 SP2T11 600 62 F	CCCTACTGCACTCTGATCTTTTG	62	SP101 SP2T11 686 714 R	CCATCTTCTGCACTGATCTGAAATAT	772
82	SP101 SP2T11 658 68 F	GAGATGTGATCACTGATGAGAGA	127	SP101 SP2T11 756 784 R	GATTCGCACTGATGATCTTCTTAA	813
83	SP101 SP2T11 776 80 F	TCCCACTCAACTGAGGATGCTG	364	SP101 SP2T11 871 896 R	GCCCACTGAGAGACTAGACGATTA	814
84	SP101 SP2T11 893 92 F	GCG	123	SP101 SP2T11 988 1012 R	CATGACGCGAGACTCTCACCACC	763

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85	SPI01_SPE111_1154_1	CAATACACACAGAGCGTGGCTGG	47	SPI01_SPE111_1251_1277_R	GAACCCACAGTGGCTTTTGTGTGTA AAACTATTTTTCAGTACTACTGAAACA C	804
86	SPI01_SPE111_1314_1	CCGCAAAATACACGCTATTATGAC	68	SPI01_SPE111_1403_1431_R	GGAATATTTTTCAGTACTACTGAAACA AG	711
87	SPI01_SPE111_1408_1	CGAGTATAGTAAATAAGATTAT GACA	67	SPI01_SPE111_1486_1515_R	GGAATATTTTTCAGTACTACTGAAACA AG	828
88	SPI01_SPE111_1688_1	CCATATTAATGTTTTCAGAAACTG GCT	60	SPI01_SPE111_1783_1808_R	ATATGATATATGTAAGTCTGGGCG	752
89	SPI01_SPE111_1711_1	CTGCTAAATCACTTGGGCAAGT	82	SPI01_SPE111_1809_1835_R	GGTGAAGCACTCTTGAATGTAACA	821
90	SPI01_SPE111_1807_1	ATGATATGTAATCAAGAAAGTCTCA CGC	33	SPI01_SPE111_1901_1927_R	TTGGACTGTATGAGTGTGTAAGTGG	1412
91	SPI01_SPE111_1967_1	TTAGCGTATGAGCGCCAGATGGG	155	SPI01_SPE111_2062_2083_R	ATATGCCAGAAATCAATCATC	755
92	SPI01_SPE111_2260_2	CAGAGACGCTTTTATGCTATCAGC	50	SPI01_SPE111_2375_2397_R	TCGTGGGTGACCTGGTGTATGA	1131
93	SPI01_SPE111_2375_2	TCATAAACAACGAGTCAACCAAG	390	SPI01_SPE111_2470_2497_R	AGCTGCTGATGATGAGCTCTGCCGTGCC	747
94	SPI01_SPE111_2468_2	ATGCGCTGCGAGAGCTCA	35	SPI01_SPE111_2543_2570_R	CCATATGATCAACGTCACATCAAGGC	770
95	SPI01_SPE111_2961_2	ACCATGACGAGAGGCAATTTGACA	15	SPI01_SPE111_3023_3045_R	GGATTTTACGACGATGAGACGC	827
96	SPI01_SPE111_3075_3	GATGACCTTTTATGATGATGTCAGC AGC	108	SPI01_SPE111_3168_3196_R	ATGCGACGACCTGTGAGAGATTTCT C	715
97	SPI01_SPE111_3386_3	AGCGTAAAGGTGAACCTT	25	SPI01_SPE111_3480_3506_R	CGACGAGTACTCTGCTCATCTTTG	769
98	SPI01_SPE111_3511_3	AGCTATGACGACGATGATGAGCAG GCA	116	SPI01_SPE111_3605_3629_R	GGTCTACACGCTGACTTGCTGATAC GC	832
111	RPOB_EC_3775_1803_F	CGACGCTGCGCTCTAC	87	RPOB_EC_3828_3858_R	CGATATAGCTGACATGAGCTGTGAT GC	797
112	VALS_EC_1433_1850_F	CGACGCTGCGCTCTAC	65	VALS_EC_1920_1943_R	GGCTTCACAGCTTTGTCAGAG	822
113	RPOB_EC_1936_1353_F	CGACGCTGCGCTCTAC	97	RPOB_EC_1438_1455_R	TTGCTCTGCGCTGGCC	1386
114	TUFB_EC_225_251_F	CGACGCTGCGCTCTAC	111	TUFB_EC_284_309_R	TATAGCACATCATCATGAGCGCAC	930
115	DNAX_EC_428_445_F	CGGCTATCTTCAAGCAGCA	72	DNAX_EC_503_522_R	CGGCTGCGCTGTGATGA	792
116	VALS_EC_1920_1943_F	CTTCTGACGACGCTGTGAGAC	85	VALS_EC_1948_1970_R	TGCGAGTTCGACGATGAGAC	1075
117	TUFB_EC_757_774_F	AGACGACGCTGCGCTGGC	6	TUFB_EC_849_867_R	GGGCTACGATCTTCAAGC	819
118	238_EC_2646_2667_F	CGTCTTCTATGTCAGAGAC	84	238_EC_2745_2765_R	TTGCTGCTGATGATGCTTTGAG	1389
119	169_EC_969_985_1P_F	AGCGAGAGCTTATCAC	19	169_EC_1061_1078_2P_R	AGGACAGGAGCTGTGAGAC	733
120	169_EC_972_985_2P_F	CGAGAGAGCTTATCAC	63	169_EC_1064_1075_2P_R	AGGACAGCTGTGAGAC	727
121	169_EC_972_985_F	CGAGAGAGCTTATCAC	63	169_EC_1064_1075_R	AGGACAGCTGTGAGAC	727
122	DNAX_EC_32_50_2_F	CGGCTATCTTCAAGCTGTGAGCTG	61	238_EC_40_59_R	AGGCTTCTTCAAGCTGTGAGCTG	740

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123	235 EC -7 15 F	GTGTGGGTTGTAAGCACTTACG	140	235 EC 430 450 R	CTATCGTCTGCTGCAAGATAT	799
124	235 EC -7 15 F	GTGTGGGTTGTAAGCACTTACG	141	235 EC 891 910 R	TTCGTCTGGGTTGGTAGTTC	1403
125	235 EC 430 450 F	ATATCTCTGTAAGCACTTACG	30	235 EC 1424 1442 R	AACTATGACTCTTCGTCTC	712
126	235 EC 891 910 F	GAATCTACCAAGCACTTACG	100	235 EC 1908 1931 R	TACTCTAAGACGGTATAGTACG	893
127	235 EC 1424 1442 F	GGACCAAGACCACTTACG	117	235 EC 2475 2494 R	CGAAACACCGCGGTGATAT	765
128	235 EC 1908 1931 F	GTCTACTATACGGTCTTACGATTA	73	235 EC 2833 2853 R	GTTTACACACCGCGGTATAC	826
129	235 EC 2475 2494 F	ATATCGACCGGGTGTTCG	31	165 EC 23 41.2 R	GGTGTGACGAGGATATTC	820
131	165 EC -60 -59 F	AGTCTGAAGGTGAGCACTTACG	28	165 EC 508 525 R	GTCTGTGACGAGGATTA	823
132	165 EC 376 345 F	GACCGGTCTCGAGCTCTTAC	95	165 EC 1041 1059 R	CAATGACGACCTCTTC	771
133	165 EC 705 724 F	GATCTGAGAGTACCGGTG	107	165 EC 1493 1512 R	ACGGTACCTTGTATACGACT	739
134	165 EC 1268 1287 F	GAGCGACACCGCACTCTATA	101	TRNA ALA--	CTCTCTGGTGTGCAAGC	780
135	165 EC 969 985 F	ACGCGAGACCACTTACC	19	165 EC 1061 1078.2 R	ACACACGAGCTGTGAGAC	719
137	165 EC 969 985 F	ACGCGAGACCACTTACC	19	165 EC 1061 1078.2 114 R	ACACACGAGCTGTGAGAC	721
138	165 EC 969 985 F	ACGCGAGACCACTTACC	19	165 EC 1061 1078.2 112 R	ACACACGAGCTGTGAGAC	718
139	165 EC 969 985 F	ACGCGAGACCACTTACC	19	165 EC 1061 1078.2 111 R	ACACACGAGCTGTGAGAC	722
140	165 EC 969 985 F	ACGCGAGACCACTTACC	19	165 EC 1061 1078.2 116 R	ACACACGAGCTGTGAGAC	720
141	165 EC 969 985 F	ACGCGAGACCACTTACC	19	165 EC 1061 1078.2 21 R	ACACACGAGCTGTGAGAC	723
142	165 EC 969 985 F	ACGCGAGACCACTTACC	19	165 EC 1061 1078.2 31 R	ACACACGAGCTGTGAGAC	724
143	165 EC 969 985 F	ACGCGAGACCACTTACC	19	165 EC 1061 1078.2 41 R	ACACACGAGCTGTGAGAC	725
147	235 EC 2452 2669 F	CTATGACGCTGAAATGCG	79	235 EC 2741 2760 R	ACTTAGAGTGTCTTACGCGT	743
156	165 EC 693 700 F	CTATGACGCTGAAATGCG	137	165 EC 880 894 R	CTACTATCCGAGGCG	796
159	165 EC 1100 1115 F	CGACGAGCTGAAATGCG	42	165 EC 1174 1188 R	TCCGACCTTCTCTC	1019
215	SSP8 BA 121 137 F	ACGCGACATGCAATGCG	3	SSP8 BA 197 216 R	TGTTGTGTTGTGAAATTC	1132
220	GR0L EC 941 959 F	TGGAAGATCTGGTGTGCG	544	GR0L EC 1039 1060 R	GAATCTGTGAGAGTATGAGC	759
221	INF8 EC 1103 1124 F	GTCTGTGAAACGAGACCTGAGA	133	INF8 EC 1174 1191 R	CATGATGTTCTACACGCG	764
222	HF18 EC 1082 1102 F	TGCGGACACTGTGACGAGAC	569	HF18 EC 1144 1168 R	CTTTCGCTCTTCTCAACTCAACAT	802
223	INF8 EC 1969 1994 F	CTTCACTGCTTAATGCTGTGAACTTAA	74	INF8 EC 2038 2058 R	AACTTCGCTCTGCTGTGATGTT	713
224	GR0L EC 215 243 F	GTGTGAAAGATTTGCTCTTAAAGC	128	GR0L EC 328 350 R	TCTAGGCTGACGAGTCAATGCG	1377
225	VALUS EC 1105 1124 F	CTTTCGCGGCTGTGTATGCGA	77	VALUS EC 1195 1214 R	ACCACTTGAATGCTGCGGTT	732
226	165 EC 556 575 F	CGGAATATCTATGCGCTTAAAG	70	165 EC 693 700 R	CGATTTATTCGCTGTACGAC	791
227	RPOC EC 1256 1277 F	ACCGAGTCTGTGACACCTTAC	16	RPOC EC 1295 1315 R	CGATTTATTCGCTGTACGAC	843
228	165 EC 774 795 F	GGAGACACGAGAGTATGATAC	122	165 EC 880 894 R	GTACTTCCCGAGGCG	796
229	RPOC EC 1584 1604 F	TGCGCCGAAAGAGTATGAGCG	567	RPOC EC 1623 1643 R	ACGCGGCACTGAGAGATGCG	737
230	165 EC 1082 1100 F	AGTTGGGTATGATGATGCG	37	165 EC 1177 1196 R	TGAATGCTATCCCACTTTCG	1158
231	165 EC 1389 1407 F	CTTGTACACATCCCGCTTC	88	165 EC 1525 1541 R	AAAGAGGTGATCCAGCG	714

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260	RNASP_EC 61_77_F	GAGGAAGTCAGCGCTC	105	RNASP_EC 345_362_R	ATAGCCCGGTTCTGTCG	751
262	RNASP_SA 31_49_F	GAGGAAGTCAGCTCTCC	103	RNASP_SA 358_379_R	ATAGCCCGGTTCTGCTCTC	750
263	16S_EC 1082_1100_F	ATGTGGGTAACTGCTCCG	37	16S_EC 1535_1541_R	AGAGAGGTATCCGCGC	714
264	16S_EC 556_575_F	CGGATTAATCGGTGGGTAAAG	70	16S_EC 774_795_R	GTATCTAATCTGTTGTCTCC	839
265	16S_EC 1082_1100_F	ATGTGGGTAACTGCTCCG	37	16S_EC 1177_1196_100_R	TGAGCTATCCGCACTCTCC	1160
266	16S_EC 1082_1100_F	ATGTGGGTAACTGCTCCG	37	16S_EC 1177_1196_100_110_R	TGAGCTATCCGCACTCTCC	1161
268	VARF_EC 511_532_F_N	GGTGTAAATAGACCTGGG	130	THRA_ALA		744
269	MOD	16S_EC 1082_1100_F	37	RHH_EC 30_49_F MOD	AGACTCTCTGCTGCAAGC	1158
270	MOD	23S_EC 2586_2607_F	203	16S_EC 1177_1196_R MOD	TGAGCTATCCGCACTCTCC	749
272	16S_EC 969_985_F	TAGACGTGCGAGCGAGTTCC	19	23S_EC 2656_2677_R MOD	AGTCTCTCCGCTCTCTCCG	807
273	16S_EC 683_700_F	ACGGGAGACCTTACC	137	16S_EC 1189_1407_R	GAGGGGCTGTGTACAG	788
274	16S_EC 49_68_F	GTGTAGCGTGAATTCG	152	16S_EC 1303_1323_R	CGAGTTGCAAGTGCAGTCG	796
275	16S_EC 49_68_F	TACACATGCACTGTAAG	152	16S_EC 880_894_R	CGTATCTCCGAGCG	734
277	CYA_BA 1349_1370_F	TACACATGCACTGTAAG	12	16S_EC 1061_1078_R	ACACAGAGCTTACGAC	800
278	F	ACACAGAGCTTACGAC	650	CYA_BA 1426_1447_R	CTTCTACATTTTACGACATAC	1159
279	16S_EC 405_432_F	TGAGTCTCCGCAAGCTGCA	464	16S_EC 1175_1196_R	TGAGCTATCCGCACTCTCC	793
280	GHOL_EC 496_516_F	TGAGTCTCCGCAAGCTGCA	34	16S_EC 507_527_R	CGCTCTCTGCGCAAGTTAG	914
281	GHOL_EC 511_536_F	ATGCGACAGTGTGCGACGAGG	8	GHOL_EC 577_596_R	TAGCCGCGCTCGAATTCAT	776
288	RPDB_EC 3802_3821_F	ATGCGACAGTGTGCGACGAGG	51	GHOL_EC 577_593_R	CGCGCTGCGAATTCAGTCTTC	786
289	RPDB_EC 3799_3821_F	CGCGCTGCGAATTCAGTCTTC	124	RPDB_EC 3862_3885_R	CGCTCTGAGGTGACATCTCTG	840
290	RPDB_EC 2146_2174_F	CGCGCTGCGAATTCAGTCTTC	52	RPDB_EC 3622_3688_R	GTCCGACTTACGCTGCTGCTCTG	736
291	APPS_EC 405_422_F	CGCGCTGCGAATTCAGTCTTC	110	RPDB_EC 2227_2245_R	ACGCTCTACGCGACGCT	738
292	APPS_EC 1374_1393_F	CGCGCTGCGAATTCAGTCTTC	69	APPS_EC 521_538_R	ACGCGACAGGTATCTGCG	811
293	TUPE_EC 957_979_F	CGCGCTGCGAATTCAGTCTTC	55	APPS_EC 1437_1455_R	GACATCGAGCTGCTGCT	829
294	16S_EC 7_33_F	CGCGCTGCGAATTCAGTCTTC	102	TUPE_EC 1034_1056_R	GGCATCACTATTTCTCTCTCTG	1345
295	VALS_EC 610_649_F	ACCGACAGGAGGACGAGC	17	16S_EC 101_122_R	TGTTACTACACCGCTCTGCGACT	929
344	16S_EC 871_890_F	CGGAGAGAACTTACCGGTC	113	VALS_EC 705_727_R	TATNACCACTGCTGAGGCTGA	736
345	16S_EC 713_732_TWOD	CGGAGAGAACTTACCGGTC	202	16S_EC 1043_1062_R	ACACACTGCACTCTCTCTC	1110
347	16S_EC 785_806_TWOD	TAGAACACCACTGCGAGGCG	560	16S_EC 789_809_TWOD_R	TGCTTGACCTACACGGGTATCTA	1278
348	16S_EC 960_981_TWOD	TGAGTATGAGACCTGCTGAGTCC	706	16S_EC 880_897_TWOD_R	TGCGCTGCTACTCTCTCTCTCTC	895

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349	238_EC_1846_1843_TM	TCTGACAGCTCCCGGTGTC	401	238_EC_1906_1924_TM0D_R	TGACCTGTATGTTACGGCC	1156
350	CALC_BA_274_303_TM0D	TGATATGTGTTTCGTGTATGCGAT	476	CALC_BA_349_376_TM0D_R	TGTACCTCTGTCTTGATTTGTATTTG	1314
351	CVA_BA_1363_1379_TM	TGGAAGTACATACAGACAGACAGAA	355	CVA_BA_1448_1467_TM0D_R	TGTGTACGGCTTCAGAGACC	1423
352	INFB_EC_1365_1393_T	TTCGTCTGTGTGCGACATACAGAT	637	INFB_EC_1439_1467_TM0D_R	TTCCTCTCTTCGATCGGTATATGCTC	1411
353	LEP_BA_756_781_TM0D	TATGCTTTGCTATATATATGAGGCA	220	LEP_BA_843_873_TM0D_R	ATCTTCAGAGATAGATTTATTTCTGT	1394
354	RPOC_EC_2218_2241_T	TCTGCGAGATATGCTGTCTGATG	405	RPOC_EC_2313_2337_TM0D_R	TGCGACCTGTGTTGATGATGATGAC	1072
355	SSPE_BA_115_137_TM0D	TGACGAGAGAGAGAGAGAGAGAGAG	255	SSPE_BA_197_222_TM0D_R	TTCGACGTCTGTTCGATGTGCAATTC	1402
356	RPLB_EC_650_679_TM0D	TGACGAGAGAGAGAGAGAGAGAGAG	449	RPLB_EC_739_762_TM0D_R	TTCGAGTGTGTTTACCCCATG	1380
357	RPLB_EC_688_710_TM0D	TGACGAGAGAGAGAGAGAGAGAGAG	296	RPLB_EC_736_757_TM0D_R	TGTCTGTGTTACCCATGAGT	1337
358	VALS_EC_1105_1124_T	TGCTGCGAGAGAGAGAGAGAGAGAG	385	VALS_EC_1195_1218_TM0D_R	TGCTGACAGACTGAGATGCGGTT	1093
359	RPOB_EC_1845_1866_T	TATGCTTCAGGAGAGAGAGAGAGAG	659	RPOB_EC_1909_1929_TM0D_R	TGCTGATGCGCTTTGCTAGG	1250
360	OD_F_2646_2667_TM	TCTGTCTTATGTCAGAGAGAGAGAG	409	238_EC_2745_2765_TM0D_R	TTCGTGCTTAGAGATGCTTAG	1434
361	16S_EC_1090_1111_2	TTTATGCTCCGAGAGAGAGAGAGAG	697	16S_EC_1175_1196_TM0D_R	TTCGAGTCTCCGACATCTCTC	1398
362	RPOB_EC_3799_3821_T	TGCGACAGCTTTCGAGAGAGAGAGAG	581	RPOB_EC_3862_3888_TM0D_R	TATCCGACTTTCGAGAGAGATTCCTG	1325
363	RPOC_EC_2146_2174_T	TGAGAGAGAGAGAGAGAGAGAGAGAG	284	RPOC_EC_2227_2245_TM0D_R	TGAGAGTCTGAGAGAGAGAGAG	898
364	RPOC_EC_1374_1393_T	TGCGAGAGAGAGAGAGAGAGAGAGAG	367	RPOC_EC_1437_1455_TM0D_R	TGAGAGTCTGAGAGAGAGAGAG	1166
367	D_F_957_979_TM0D	TGCGAGAGAGAGAGAGAGAGAGAGAG	308	TUFB_EC_1034_1058_TM0D_R	TGAGAGTCTGAGAGAGAGAGAG	1276
423	SP101_SPT11_893_92	TGCGAGAGAGAGAGAGAGAGAGAGAG	580	SP101_SPT11_908_1012_TM0D	TGAGAGTCTGAGAGAGAGAGAG	990
424	SP101_SPT11_1151_1	TGCGAGAGAGAGAGAGAGAGAGAGAG	258	OD_R_SPT11_1251_1277_TM	TGAGAGTCTGAGAGAGAGAGAG	1155
425	SP101_SPT11_118_14	TGCGAGAGAGAGAGAGAGAGAGAGAG	528	OD_R_SPT11_213_238_TM0D	TGAGAGTCTGAGAGAGAGAGAG	1422
426	SP101_SPT11_1314_1	TGCGAGAGAGAGAGAGAGAGAGAGAG	363	SP101_SPT11_1402_1431_TM	TGAGAGTCTGAGAGAGAGAGAG	849
427	437_TM0D_F	TGCGAGAGAGAGAGAGAGAGAGAGAG	359	OD_R_1486_1515_TM	TGAGAGTCTGAGAGAGAGAGAG	1268

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428	SP101_SPT11_1698_1 6_TMOD_F	TCTGTAATATATGCTTTACGAAACT GCGT	334	SP101_SPT11_1793_1808_TM OD_R	TATATATATATATGAACTGCGCG A	932
429	SP101_SPT11_1711_1 733_TMOD_F	TCTGCTAAATATGCGCAAGGT TATGATATCAATTCAGAGAGCTTC	406	SP101_SPT11_1808_1835_TM OD_R	TCTGTAAGAGACTCTTGAATGTATTC	1239
430	SP101_SPT11_1807_1 835_TMOD_F	ACGC	235	SP101_SPT11_1901_1927_TM OD_R	TTTGGACTGTATATCACTGAACTACTG	1439
431	SP101_SPT11_1967_1 951_TMOD_F	TTTAAAGCTTATCTGCGCCAGATGCG TACGAGCTGTGAAATCGCGCAAG	649	SP101_SPT11_2062_2083_TM OD_R	TATGCGCCGAAATCAATCATC	940
432	SP101_SPT11_216_24 3_TMOD_F	ATT	210	SP101_SPT11_308_333_TMOD R	TTTCCACTTTTACACTCTCTTGTCTG	1404
433	SP101_SPT11_2260_2 283_TMOD_F	TCTGAGAGACCTTTATCTATACAC	272	SP101_SPT11_2375_2397_TM OD_R	TTCTGAGAGAGCTGCTTTTAA	1393
434	SP101_SPT11_2375_2 399_TMOD_F	TTCTAAACACCGTCTACCGAGAG	675	SP101_SPT11_2470_2497_TM OD_R	TACTCTCAATGAGCTTTCTCATGCG	918
435	SP101_SPT11_2468_2 487_TMOD_F	TATGAGCACTGCGGAGCTCA	238	SP101_SPT11_2543_2570_TM OD_R	CTCTTAAGGTCACTCACTCAAG	1007
436	SP101_SPT11_266_29 5_TMOD_F	TCTGTACTTCTGATCTCAAGGCTG	417	SP101_SPT11_355_380_TMOD R	TCTGCTTTGATGCTGATCCCTTC	1249
437	SP101_SPT11_2961_2 984_TMOD_F	TATCATGACAGAGAGCTTTTACCA	183	SP101_SPT11_3023_3045_TM OD_R	TGGAATTTACCGCACTTACAGACC	1264
438	SP101_SPT11_3075_3 103_TMOD_F	TGATGATCTTTTATCTATGTCAGG	473	SP101_SPT11_3168_3196_TM OD_R	TATCTGAGACCACTTGTGAAGATTC	875
439	SP101_SPT11_322_34 4_TMOD_F	TATCAAAATGTCGCTTTTATCTGAC	631	SP101_SPT11_423_441_TMOD R	TATCCCTGCTCTCTGCTGCG	934
440	SP101_SPT11_3386_3 403_TMOD_F	TACTGTAAAGTGTAACTCTT	215	SP101_SPT11_3480_3506_TM OD_R	TCCGAGCACTTACTGCTGCTCACTTGTG	1005
441	SP101_SPT11_3811_3 TACTGTAAAGTGTAACTCTT	531	SP101_SPT11_3605_3623_TM OD_R	SP101_SPT11_448_473_TMOD R	TGCGTCTCACTCTGCTCTGATAC	1294
442	SP101_SPT11_358_38 7_TMOD_F	TGAGAC	588	SP101_SPT11_686_714_TMOD R	TCCAACTTTTCAACAGAGATCAGC	998
443	SP101_SPT11_600_62 9_TMOD_F	TCTGTACTTCTGATCTATGATCTTTT	348	SP101_SPT11_756_784_TMOD R	TCCCACTTTTCACTCACTCTGAGAA	1018
444	SP101_SPT11_658_68 4_TMOD_F	TCTGATGATGATCTCACTTAAAGAG	589	SP101_SPT11_871_896_TMOD R	TGATTTGGCATTAAGTATTTTCTAA	1189
445	SP101_SPT11_776_80 1_TMOD_F	TTTGCCTCACTCAAACTTAGGAAATGG	673	SP101_SPT11_92_116_TMOD R	TCCCACTCAAGAACTTACAGATTA	1217
446	SP101_SPT11_1_29_T MOD_F	TAACTCTATTATTGAAABACCACTAG	154	SP101_SPT11_3170_3194_R SP101_SPT11_448_473_R	TCTTACCACTGCTTCAAGAGCAG	1044
447	SP101_SPT11_364_38 104_F	TGAGCACTCAAGCAGCTATTG	276	SP101_SPT11_3170_3194_R SP101_SPT11_3170_3194_R	TACTTTTCAACACAGAGATCAGC	894
448	SP101_SPT11_3085_3 104_F	TAGTATATGCTGCGGAGCC	216	SP101_SPT11_3170_3194_R SP101_SPT11_3170_3194_R	TCTGAGAGCACTTGTGAAAGATTC	1066
449	SP101_SPT11_3085_3 104_F	TCTCAAGCTGTGTGTAAAGG	309	SP101_SPT11_3170_3194_R SP101_SPT11_3170_3194_R	TGAGCTGTGCTTACCGAGGAG	1336
481	SP101_SPT11_3085_3 104_F	TATGCTCTTACTCA	239	SP101_SPT11_3170_3194_R SP101_SPT11_3170_3194_R	TGTACTCTGCTGAGT	1346

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482	BOWT_X52066_538_55	BA*TPSGC*TP*CP*TPA*CP*TP*C BA	143	BOWT_X52066_647_660P_R	TP*TPA*CP*TPA*CP*TPSGC	1146
483	BOWT_X52066_701_72	GAATGACANTATCAAAAT	94	BOWT_X52066_759_775_R	TPACTCTPACCOMCTC	1367
484	BOWT_X52066_701_72	GA*TPAG*CPAA*TP*TPAA*TP*C P*CPAAAT	91	BOWT_X52066_759_775P_R	TPA*CP*TP*CP*TPAA*CP*CP*C PA*CP*TPC	1359
485	BOWT_X52066_450_47	TCRTGTAAATATGACCTGACC	393	BOWT_X52066_517_533_R	TPACCAATTCGCTGAGTCAA	859
486	BOWT_X52066_450_47	TP*CP*TPAGTAATATGAGA*CP*CP *CP*TP*CGACC	142	BOWT_X52066_517_539P_R	TPACCA*TP*TP*TP*CPGCTGAGAT P*TP*CPAA	857
487	BOWT_X52066_591_62	TGATCTACTGATGATGATCAAMTC CTCT	463	BOWT_X52066_644_671_R	TCATGTCTTAATCTCTCTGCTGATCTG	992
608	SSPE BA 156 168P_F	TGCTP5CP*TPAGCAAT	616	SSPE BA 243_255P_R	TCCAGCTGATGATPCT	1241
609	SSPE BA 75 89P_F	TPCQAGATGTP*TPCGACC	192	SSPE BA 163_177P_R	TGCGCTP*TP*GAATGCT	1336
610	SSPE BA 150 168P_F	TGCTTCTGCTP5CP*TPAGCAAT	533	SSPE BA 243_264P_R	TGATGCTTCTGCTGCTGATCTG	1191
611	SSPE BA 72 89P_F	TGCTTCTGCTP5CP*TPAGCAAT	602	SSPE BA 163_182P_R	TCATGCTTCTGCTGCTGATCTG	995
612	SSPE BA 114 137P_F	TGCTTCTGCTP5CP*TPAGCAAT	255	SSPE BA 196_222P_R	TCATGCTTCTGCTGCTGATCTG	1401
699	SSPE BA 123 153_F	TGCTTCTGCTP5CP*TPAGCAAT	488	SSPE BA 202_231_R	TCATGCTTCTGCTGCTGATCTG	1431
700	SSPE BA 156 168_F	TGCTTCTGCTP5CP*TPAGCAAT	612	SSPE BA 243_255_R	TCATGCTTCTGCTGCTGATCTG	1302
701	SSPE BA 75 89_F	TGCTTCTGCTP5CP*TPAGCAAT	179	SSPE BA 163_177_R	TCATGCTTCTGCTGCTGATCTG	1338
702	SSPE BA 150 168_F	TGCTTCTGCTP5CP*TPAGCAAT	533	SSPE BA 243_264_R	TCATGCTTCTGCTGCTGATCTG	1190
703	SSPE BA 72 89_F	TGCTTCTGCTP5CP*TPAGCAAT	600	SSPE BA 163_182_R	TCATGCTTCTGCTGCTGATCTG	995
704	SSPE BA 146 163_F	TGCTTCTGCTP5CP*TPAGCAAT	484	SSPE BA 242_267_R	TCATGCTTCTGCTGCTGATCTG	1421
705	SSPE BA 63 89_F	TGCTTCTGCTP5CP*TPAGCAAT	518	SSPE BA 163_191_R	TCATGCTTCTGCTGCTGATCTG	986
706	SSPE BA 114 137_F	TGCTTCTGCTP5CP*TPAGCAAT	255	SSPE BA 196_222_R	TCATGCTTCTGCTGCTGATCTG	1402
770	PLA_A053945_7377_7	TGCTTCTGCTP5CP*TPAGCAAT	442	PLA_A053945_7434_7462_R	TCATGCTTCTGCTGCTGATCTG	1313
771	PLA_A053945_7380_7	TGCTTCTGCTP5CP*TPAGCAAT	327	PLA_A053945_7482_7502_R	TCATGCTTCTGCTGCTGATCTG	1304
772	PLA_A053945_7481_7	TGCTTCTGCTP5CP*TPAGCAAT	481	PLA_A053945_7539_7562_R	TCATGCTTCTGCTGCTGATCTG	943
773	PLA_A053945_7486_7	TGCTTCTGCTP5CP*TPAGCAAT	657	PLA_A053945_7575_7600_R	TCATGCTTCTGCTGCTGATCTG	879
774	CAPI_A053947_33407	TGCTTCTGCTP5CP*TPAGCAAT	292	CAPI_A053947_33494_33511	TCATGCTTCTGCTGCTGATCTG	1235
775	CAPI_A053947_33515	TGCTTCTGCTP5CP*TPAGCAAT	270	CAPI_A053947_33595_33621	TCATGCTTCTGCTGCTGATCTG	1053
776	CAPI_A053947_33435	TGCTTCTGCTP5CP*TPAGCAAT	542	CAPI_A053947_33495_33517	TCATGCTTCTGCTGCTGATCTG	1183

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777	CNFI_A003947_33607 33716_F	TGAGGTGGAATTAACCAACATCA CTGC	286	CNFI_A003947_33755_33782 R	TCAGGTCTCTACCGTTTACTTACGAG	962
778	INV_U22457_515_539_	TGCTCCCTGTATATGACTCTGCTTC	573	INV_U22457_571_598_R	TGTTAAGTGTGTGCGGTGTCTTTAT	1343
779	INV_U22457_699_724_	TGCTGAGGCTGAGCACTATATATAC	525	INV_U22457_753_776_R	TUNCGCGAGGTCCATCCATG	976
780	INV_U22457_834_850_	TTATTATCTCTGACTCCCAACTG	664	INV_U22457_942_966_R	TGACCCAGGCTGAAGCTTACTG	1154
781	INV_U22457_1058_1088_	TGTTAACTGAGCTTTATAGGCGCA	597	INV_U22457_1619_1643_R	TGCTGTTCAGATTAATCTTACAA	1408
782	LL_NC003143_2366996	TGAGCGCTGAGCACTACATCTC	637	LL_NC003143_2367073_23670	TCTCATCCGATTAATGCGCATCA	1123
783	LL_NC003143_2367172	TGAGCGCACTGAGCACTCTAC	550	LL_NC003143_2367249_23672	TGCGCAAGCTCTACACCTTTGG	1272
784	RELB_EC_649_679_F	TGCGCTGCTGTTGGGCTCTGTAAT	620	RELB_EC_733_762_TM0D_R	TTCCAGTGTCTGTTTTACCCCTGG	1380
785	RELB_EC_642_6799_F	TGCGCTGTGTGTGTGTGCTGTTT	646	RELB_EC_733_762_TM0D_R	TTCCAGTGTCTGTTTTACCCCTGG	1380
786	MECA_Y14051_3311_3	TGAGCTGTGTAATGAC	653	MECA_Y14051_3367_3393_R	TGCGATGCGAGGTGTAGAGGTGTA	1333
787	MECA_Y14051_3774_38	TGAGCACTGCTGTGAGCACTGACTG	144	MECA_Y14051_3828_3854_R	TCCGATCTAGCTTCCACATACCT	1015
788	MECA_Y14051_3774_38	TGAGCACTGCTGTGAGCACTGACTG	434	MECA_Y14051_3690_3719_R	TGATCTGTAATGTTTATCTTTACGC	1181
789	MECA_Y14051_3645_36	TGAGCACTGCTGTGAGCACTGACTG	288	MECA_Y14051_4555_4581_R	TGCGTAGAGTCAATGAGGTGTGCT	1269
790	MECA_Y14051_4507_45	TGAGCACTGCTGTGAGCACTGACTG	626	MECA_Y14051_4586_4610_R	TATCTCTGTTACTGATCCATCA	939
791	MECA_Y14051_4510_45	TGAGCACTGCTGTGAGCACTGACTG	262	MECA_Y14051_4765_4793_R	TACACCCCGAGATTAATCTTTTCC	858
792	MECA_Y14051_4669_46	TGAGCACTGCTGTGAGCACTGACTG	389	MECA_Y14051_4520_4600_R	TGCGTGTGTGTGTGTGTGTGT	1357
793	MECA_Y14051_4520_45	TGAGCACTGCTGTGAGCACTGACTG	389	MECA_Y14051_4600_4610_R	TGCGTGTGTGTGTGTGTGTGT	1358
794	TRPE_AY094355_1467_	TGAGCACTGCTGTGAGCACTGACTG	36	TRPE_AY094355_1569_1592_R	TGCGCGAGCTTTTATTTGGTTTC	1231
795	TRPE_AY094355_1445_	TGAGCACTGCTGTGAGCACTGACTG	557	TRPE_AY094355_1551_1580_R	TATTTGGTTTCTTCACTGATCT	944
796	TRPE_AY094355_1471_F	TGAGCACTGCTGTGAGCACTGACTG	247	TRPE_AY094355_1392_1418_R	TGCTCTTTTCCAGGCTCTACTCATC	1040
797	TRPE_AY094355_1278_	TGAGCACTGCTGTGAGCACTGACTG	357	TRPE_AY094355_1171_1196_R	TGAGTGTGTGCGCCAGTATCA	885
798	TRPE_AY094355_1064_	TGAGCACTGCTGTGAGCACTGACTG	135	TRPE_AY094355_769_791_R	TGCGAAATCGAGGAGCTG	1372
799	TRPE_AY094355_666_	TGAGCACTGCTGTGAGCACTGACTG	483	TRPE_AY094355_864_883_R	TGCGAGTCACTGCT	1218
800	TRPE_AY094355_757_7	TGAGCACTGCTGTGAGCACTGACTG				

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1055	CJST_CJ_2869_2895_F	TKAGCTGTGTCTTACAGAGAGATTC	432	TCCGCTGTGTCTTCAAAAGCATTTTT	A	1045
1056	CJST_CJ_1880_1910_P	KCCGATATATCTGCGATTTTCCCA	317	TGCTTCTACTGTCTTGCATTAAGTTT	CCA	1309
1057	CJST_CJ_2185_2212_F	TKAGNAAAAGAGCGAGTGGCTAT	208	TATAGT	TGATAGT	1152
1058	CJST_CJ_1643_1670_F	TTATGCTTTGTGGAGCTATAGTCTAT	660	TGCGATATGTGTCTATGTCAGCAAAAGA	F	1198
1059	CJST_CJ_2165_2194_F	TGCGAGATCTTGTGTGGTGTAGATG	511	TCCGACAGCTGGATATTAAGTCTGTGTT	CCGCT	1002
1060	CJST_CJ_599_632_F	TGAAAATAATCCAGAGACATFAGCA	424	TCCGACAAAGATGATGATCACTATT	TTTAC	1024
1061	CJST_CJ_360_393_F	TCTGTATATCTCGATATGATATATTC	345	TACAGATCTTCAAAACATTAAGCTGT	AATTTTC	882
1062	CJST_CJ_2678_2703_F	TCCCGACAGCTCGATGAATTTCAAC	321	TGTCGCTTTTGTCTGTCATACAAAGC	ATTTGCT	1319
1063	CJST_CJ_1268_1299_F	AGTTATAAACAGAGCTTCTCTATGAC	29	TGCGTTTAAGCTACTATGATCAAGAG	ATA	1096
1064	CJST_CJ_1580_1713_P	TCCTCTCTCTATATATAGAAATTC	479	TATGTAGTATGATCTTACTACATAGAC		938
1065	CJST_CJ_2857_2887_F	TCGATCTCTTAAGAGCTGTGTCTT	565	TGCTTCAAAAGCATTTTACATTTTCG	TTAAG	1253
1070	RNASEP_BKM_560_599_P	TACCA	512	RNASEP_BKM_665_696_R	TCCGAAAGACCGATATCTGTGTC	1034
1071	RNASEP_BKM_616_637_P	TCCGATAGAGATGCTGTCCAGC	333	RNASEP_BKM_655_687_R	TGCCGATAGACCGATATCTGTGTC	1222
1072	RNASEP_BDP_574_592_P	TGCGACAGCGCATCTCCGTG	561	RNASEP_BDP_616_635_R	TGCTTTCAACCGTGTCAATCCG	1115
1073	23S_BKM_1110_1129_P	TCCCGAAGATATTAACAGG	510	23S_BKM_1176_1201_R	TCCGACGATCTGCTGCTCTCTCA	1074
1074	23S_BKM_515_536_F	TGCTATCAAGATCCGAGAGCT	496	23S_BKM_615_635_R	TCCGACCTGCTTCTGCTTAG	1088
1075	RNASEP_CLB_459_487_F	TACAGATGTGTCAGAGATATATACC	162	RNASEP_CLB_498_526_R	TGCTTCTACTTACCGCTTGTCAACCTTAC	1247
1076	RNASEP_CLB_459_487_P	TACAGATGTGTCAGAGATATATACC	162	RNASEP_CLB_498_522_R	TTTACTCTCGCTTTTCAACCGCTTACC	1426
1077	ICD_CXB_93_120_F	TCTGTAGACAGCAATATATCCGTTTA	343	ICD_CXB_172_194_R	TAGAGATTTTTCACCGCGCATC	921
1078	ICD_CXB_92_120_P	ATCTGTACGACACCAATATTCGTTT	671	ICD_CXB_172_194_R	TAGATATTTTTCACCGCGCGATC	921
1079	ICD_CXB_176_196_P	TGCTGTGTGAAGATATCTAGCT	369	ICD_CXB_224_247_R	TAGCTTTTCTTCGCGCTAGATCT	916
1080	66_68_1311_NC002971_60	TCGATATATATCCACATCCAGCGCTC	290	1811111_NC002971_6928_6954_R	TTAAGCTCGGATACATATGTTGCGTC	848
1081	66_68_1311_NC002971_74	TCGATATATATCCACATATATATGCT	594	1811111_NC002971_7528_7554_R	TGATACAGACTCTCTTTTTCGATCT	952
1082	RNASEP_RKP_419_448_P	TGGTATAGAGCGAACCGTAAATGCT	599	RNASEP_RKP_542_565_R	TATAGATCTTACCCGATATCAAA	957

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1083	RNASP_RFP_422_443_F	TACAGGCGACGCGTAACTGG	159	RNASP_RFP_542_565_R	TGAGCGATCTACCGCATACA	957
1084	RNASP_RFP_466_491_F	TCCACDAGCGAGATCAATAGCC	310	RNASP_RFP_542_565_R	TGAGCGATCTACCGCATACA	957
1085	RNASP_RFP_264_287_F	TCTAAATGCTGTGAGTGGTG	391	RNASP_RFP_295_321_R	TCTAGAGTCGGACTTCTCGTGA	1119
1086	RNASP_RFP_426_448_F	TGCAACCGGTAAAGTGACACA	497	RNASP_RFP_542_565_R	TGAGCGATCTACCGCATACA	957
1087	OMP_RFP_860_890_F	TTACAGGAGTTTGAAGTGAATCTA	654	OMP_RFP_972_996_R	TCTCGAGCTCTACTCTCTCATTA	1051
1088	OMP_RFP_1192_1221_F	TCTACTGATTTTGGTAATCTCGAC	392	OMP_RFP_1288_1315_R	TAGACAAAGTTATACGACTGCGAT	910
1089	OMP_RFP_3417_3440_F	TGCAAGTGGTATCTCAACGTGGG	485	OMP_RFP_3520_3550_R	TGGTGTGATCTCTGTAGTGTGGCAT	1310
1090	GLTA_RFP_1043_1072_F	TGGACTTGAGACTATCTCTTAA	576	GLTA_RFP_1138_1162_R	TGACATTTGCGAGGTATACCGCT	1147
1091	GLTA_RFP_400_428_F	TCTCTCTCTCTCTCTCTCTCTCTCT	413	GLTA_RFP_499_529_R	TGCTGGGTATCTTAGAGCATCATCTAAT	1305
1092	GLTA_RFP_1023_1055_F	TGCAAGCTTGAGCATATGCTCTTAA	330	GLTA_RFP_1129_1156_R	TTGCGAGCGTTATACCGTCTTATA	1415
1093	GLTA_RFP_1043_1072_F	TGCAAGCTTGAGCATATGCTCTTAA	553	GLTA_RFP_1138_1162_R	TGACATTTGCGAGGTATACCGCT	1147
1094	GLTA_RFP_1043_1072_F	TGCAAGCTTGAGCATATGCTCTTAA	543	GLTA_RFP_1138_1162_R	TGCAAGCTTGCGAGGTATACCGCT	1330
1095	GLTA_RFP_400_428_F	TCTCTCTCTCTCTCTCTCTCTCTCT	413	GLTA_RFP_505_534_R	TGCAAGCTTGAGGTATCTTAGCGATCT	1230
1096	CTTA_VBC_117_142_F	TCTTATCTCGAGGAGGAGAGTGT	410	CTTA_VBC_194_218_R	TGCTTACACATCTCTGTGATTC	1226
1097	CTTA_VBC_351_377_F	TGATATAGGCGATGAGTCTGATC	630	CTTA_VBC_441_466_R	TGTCATCAAGCACCCCAATATGACT	1324
1098	RNASP_VBC_331_349_F	TGCGCGATCTGATGGT	325	RNASP_VBC_388_414_R	TGACTTCTCTCTCTCTCTCTCTCT	1163
1099	TOX_VBC_135_158_F	TCTTATAGGCGAGGAGGAGGAGG	362	TOX_VBC_221_246_R	TGCAAGCTTGCTCTGCGACACA	1370
1100	ASD_FRT_1_29_F	TCTTATAGGCGATGATTTATGTTG	690	ASD_FRT_86_116_R	TGAGATCTGCAAAAGAGTGGCAAA	1156
1101	ASD_FRT_43_76_F	TGCAAGTGAATCTCTCTATATGCA	295	ASD_FRT_129_156_R	TGCTTATGTTGCTATAACTGTGGC	1009
1102	GALE_FRT_168_199_F	TTATGCTAGACTTTTAGTAAG	658	GALE_FRT_241_269_R	TGACTTACAGCTTAAAGCAGCAAT	973
1103	GALE_FRT_834_865_F	TCGAAAGCGCTTAGTATAGAGATTC	245	GALE_FRT_901_925_R	TGACTTGGCAGCTATCGCAAACT	915
1104	GALE_FRT_308_339_F	TGCAAGTCACTAACTTACTTONG	306	GALE_FRT_390_422_R	TCTCTGTAAGAGGTGGTTATTTCTA	1136
1105	TPAH_SGF_258_277_F	CTATG	458	TPAH_SGF_301_327_R	TOCA	1085
1106	TPAH_SGF_113_134_F	TGAGGAGCGCTCTGCGCTCA	350	TPAH_SGF_172_191_R	TCTTCTATGCTCTATAGAGCAGGAG	1441

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1107	IPAH SQF 462 486 F	TGACGACGCTTCGCGAGACCTT	271	IPAH SQF 522 540 R	TGTCNCTCCGACACGCCA	1322
1111	RMASBP BHM 467 486 F	TGACCCCTTCGCGAGACGCGAA	147	RMASBP BHM 542 561 R	TGCTTCGCGACGCTTACCGCG	1227
1112	RMASBP BHM 325 347 F	TACCCGAGCGAGTTCGACGCA	185	RMASBP BHM 402 428 R	TGCTTACCCGCGCTTTCACCTTAC	1125
1128	HUPB CJ 113 134 F	TAGTTCGCTCCACAGCTTGCGCT	230	HUPB CJ 157 188 R	TGCTTATGTTGAGATGCTGACGCG	1028
1129	HUPB CJ 76 102 F	AT	324	HUPB CJ 157 188 R	TGCGTAATGATGAACTTACGTCAG	1028
1130	HUPB CJ 76 102 F	AT	324	HUPB CJ 114 135 R	TAGCCNACCTGTTTGGCAACT	913
1151	AB MUST-11- OIF007 62 91 F	TGAGATTCGCTGACCTTTTATCTGAA	454	AB MUST-11- OIF007 169 203 R	TGTACNATTTGAAACATATGATGACA	1418
1152	AB MUST-11- OIF007 195 214 F	TATGTTTTCANATGTCAGGCTGAG	243	AB MUST-11- OIF007 291 324 R	TGACAGGTTCTCTTCTTANTATATTTTC	969
1153	AB MUST-11- OIF007 230 289 F	TGCGACGCTTACAGGCTGCCGAAA	541	AB MUST-11- OIF007 364 393 R	TGCGNATGCAATATGATATTTGACGAG	1400
1154	AB MUST-11- OIF007 206 239 F	TGAGTTCGCTGATGATGATGATGAC	436	AB MUST-11- OIF007 318 344 R	TGCGCGAAAACCTCCCTTTTCAGCG	1036
1155	AB MUST-11- OIF007 522 552 F	TGAGTTCGCTGATGATGATGATGAC	378	AB MUST-11- OIF007 597 610 R	TTCNCTTCGAGGATGATGTCGTCG	1392
1156	AB MUST-11- OIF007 547 571 F	TGAGTTCGCTGATGATGATGATGAC	250	AB MUST-11- OIF007 656 686 R	TGCTTCTACGATTTCTTCTACGCTAC	902
1157	AB MUST-11- OIF007 601 627 F	TGAGTTCGCTGATGATGATGATGAC	256	AB MUST-11- OIF007 710 736 R	TATGCGGCGGTAGTGCATCATCTTCTC	881
1158	AB MUST-11- OIF007 1202 1235 F	TGAGTTCGCTGATGATGATGATGAC	384	AB MUST-11- OIF007 1266 1296 R	TAG	878
1159	AB MUST-11- OIF007 1212 1235 P	TGAGTTCGCTGATGATGATGATGAC	384	AB MUST-11- OIF007 1299 1315 R	TGCACTTCGCTGCTGACGCG	1199
1160	AB MUST-11- OIF007 1214 1264 F	TGAGTTCGCTGATGATGATGATGAC	694	AB MUST-11- OIF007 1335 1362 R	TGCACTTCGCTGCTGACGCG	1215
1161	AB MUST-11- OIF007 1327 1356 F	TGAGTTCGCTGATGATGATGATGAC	225	AB MUST-11- OIF007 1422 1448 R	TGCGAGTTTCGATTCATTCAGTTCGTC	1212
1162	AB MUST-11- OIF007 1345 1359 F	TGAGTTCGCTGATGATGATGATGAC	393	AB MUST-11- OIF007 1470 1494 R	TGCGAGTTTCGATTCATTCAGTTCGTC	1083
1163	AB MUST-11- OIF007 1351 1375 F	TGAGTTCGCTGATGATGATGATGAC	662	AB MUST-11- OIF007 1470 1494 R	TGCGAGTTTCGATTCATTCAGTTCGTC	1083
1164	AB MUST-11- OIF007 1387 1412 F	TGAGTTCGCTGATGATGATGATGAC	422	AB MUST-11- OIF007 1470 1494 R	TGCGAGTTTCGATTCATTCAGTTCGTC	1083
1165	AB MUST-11- OIF007 1412 1459 F	TGAGTTCGCTGATGATGATGATGAC	194	AB MUST-11- OIF007 1470 1494 R	TGCGAGTTTCGATTCATTCAGTTCGTC	1173
1166	AB MUST-11- OIF007 1456 1593 F	TGAGTTCGCTGATGATGATGATGAC	684	AB MUST-11- OIF007 1470 1494 R	TGCGAGTTTCGATTCATTCAGTTCGTC	1173
1167	AB MUST-11- OIF007 1611 1638 F	TGAGTTCGCTGATGATGATGATGAC	375	AB MUST-11- OIF007 1731 1757 R	TGCGAGTTTCGATTCATTCAGTTCGTC	890

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1168	AB_MUST-11- 01F007_1726_1732_F	TACACATATTAATGCTGCTGCTCTT C	182	AB_MUST-11- 01F007_1730_1821_R	TGACATGACATAGATGCTGCTGAGTAT AAGC	1195
1169	AB_MUST-11- 01F007_1792_1826_F	TATTAATGATTCGCAATCATTTCAAT TCTCTTGCTG	656	AB_MUST-11- 01F007_1876_1909_R	TGATATTCGAGAGATGATCAATTTTC TGACGA	1151
	AB_MUST-11- 01F007_1792_1826_F	TATTAATGATTCGCAATCATTTCAAT TCTCTTGCTG	656	AB_MUST-11- 01F007_1895_1927_R	TGCGTGTATCATCAATGAGATATATGC AAGAA	1224
1171	AB_MUST-11- 01F007_1970_2002_F	TGTTATATGACCAATATCTTTTTCG AAGTGTG	618	AB_MUST-11- 01F007_2097_2118_R	TGACGCGCTGATGACACACGCTC TGACGCGCTGACACACACGCG	1157
1172	RMSHP_BOX_461_468 GXSH_NC002505_46_70	TAMCCCGCATCGGAGCGAAACCGAA TA	147	RMSHP_BOX_542_561_2_R	TGACGCGCTGACACACACGCG ATC	1228
2000	FUR_NC002505_87_113	TGACGCTGATGACGATGAGATCTGCTC TGACGCTGATGACGATGAGATCTGCTC	278	CTXB_NC002505_132_162_R	TGCGCTGATGATTTGCTGATGACGAT ATC	1039
2001	FUR_NC002505_87_113	TGACGCTGATGACGATGAGATCTGCTC TGACGCTGATGACGATGAGATCTGCTC	465	FUR_NC002505_205_228_R	TGCGCTGATGATTTGCTGCGAGAT ATC	1037
2002	FUR_NC002505_87_113	TGACGCTGATGACGATGAGATCTGCTC TGACGCTGATGACGATGAGATCTGCTC	465	FUR_NC002505_178_205_R	TGACGATATCTGCTGATCATCAATATGCTT AT	974
2003	GAPA_NC002505_331_5	TGACGACACATTTATCTATGCTGTG TG	356	GAPA_NC002505_646_671_R	TGACATCATGATCGCAATATGCTCATC AT	980
2004	GAPA_NC002505_694_7	TGATATGAGGCGACACAGCATATGTA TG	259	GAPA_NC002505_769_799_R	TGACATCATGATCGCAATATGCTCATC AT	1046
2005	GAPA_NC002505_751_7	TGCTGTATCATCTCATTTCCGCTGTG ATAC	517	GAPA_NC002505_856_881_R	TGACATCATGATCGCAATATGCTGTG AT	1011
2006	GAPA_NC002505_2_32	TGCGCGCAATATGATCATTCATCATGAT TG	501	GATB_NC002505_199_225_R	TGCGACCATCGAATGATGCTGTG AT	1003
2007	GATB_NC002505_123_1	TGATGATGATGATGATGATGATGATGAT ATAC	460	GATB_NC002505_199_225_R	TGCGATCATGCTGATGATGATGATGATGAT AT	1042
2008	GATB_NC002505_768_7	TATGCTGTGATGATGATGATGATGATGAT AT	236	GATB_NC002505_832_860_R	TGGAACACCGCTGATGATGATGATGATGAT C	1262
2009	GATB_NC002505_837_8	TGATGATGATGATGATGATGATGATGAT AT	603	GATB_NC002505_937_957_R	TGCTTCACGCTGATGATGATGATGATGAT AT	1054
2010	GATB_NC002505_934_9	TGCGCTGTGATGATGATGATGATGATGAT AT	377	GATB_NC002505_982_1007_R	TGCGTTTGATGATGATGATGATGATGATGAT AT	1283
2011	GATB_NC002505_1161_1	TAAAGCGCTGATGATGATGATGATGATGAT AT	148	GATB_NC002505_1255_1284_R	TGATGATGATGATGATGATGATGATGATGAT AT	1172
2012	OPU_NC002505_85_11	TACGCTGTGATGATGATGATGATGATGAT AT	190	OPU_NC002505_154_180_R	TGCTTCGATGATGATGATGATGATGATGAT AT	1254
2013	OPU_NC002505_358_2	TGACGCTGATGATGATGATGATGATGATGAT AT	451	OPU_NC002505_346_369_R	TGCGACACCGCTGATGATGATGATGATGAT AT	1033
2014	OPU_NC002505_431_4	TGACGCTGATGATGATGATGATGATGATGAT AT	266	OPU_NC002505_544_567_R	TGCTTCGATGATGATGATGATGATGATGAT AT	1094
2015	OPU_NC002505_331_5	TGACGCTGATGATGATGATGATGATGATGAT AT	223	OPU_NC002505_625_651_R	TGACGATGATGATGATGATGATGATGATGAT AT	908
2016	OPU_NC002505_727_7	TGACGCTGATGATGATGATGATGATGATGAT AT	234	OPU_NC002505_725_751_R	TGCGCTGATGATGATGATGATGATGATGAT AT	1291
2017	OPU_NC002505_431_4	TGACGCTGATGATGATGATGATGATGATGAT AT	181	OPU_NC002505_811_835_R	TGCGCTGATGATGATGATGATGATGATGAT AT	911

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47_F	OMPU NC002505_931_9	TTACTACTTACGCGAAGCTCG	193	OMPU NC002505_1033_1053_R	TTAGAGTCTTAACTGAGACC	1368
2018	53_F	TTACTACTTCTTAAAGCGGACTTTC	197	OMPU NC002505_1033_1054_R	TGTTAGAGTCTGTTAGCTGAGACC	1307
2019	238_F	TCGCTGATCTTCTTAAAGCGGACTTTC	269	TCFA NC002505_148_170_R	TTCTGGAGTCTTAACTGCGCTG	1391
2020	238_F	TCGCTGATCTTCTTAAAGCGGACTTTC	574	TDH NC004605_357_386_R	TTCTGGAGTCTTAACTGCGCTG	1351
2021	TDH NC004605_265_28	TCGCTGATCTTCTTAAAGCGGACTTTC	412	VYFA NC004460_852_886_R	TGCTGAGTCTTAACTGCGCTG	887
2022	42_F	TCGCTGATCTTCTTAAAGCGGACTTTC	508	235_EC_2746_2770_R	TGCTGAGTCTTAACTGCGCTG	1297
2023	235_EC_2643_2667_F	TGCTGATCTTCTTAAAGCGGACTTTC	202	165_EC_789_811_R	TGCTGAGTCTTAACTGCGCTG	1240
2024	165_EC_713_732_TM0D	TGCTGATCTTCTTAAAGCGGACTTTC	560	165_EC_880_897_TM0D_R	TGCTGAGTCTTAACTGCGCTG	1278
2025	165_EC_784_806_F	TGCTGATCTTCTTAAAGCGGACTTTC	634	165_EC_1052_1074_R	TGCTGAGTCTTAACTGCGCTG	896
2026	165_EC_959_981_F	TGCTGATCTTCTTAAAGCGGACTTTC	489	TUFB_EC_1034_1058_2_R	TGCTGAGTCTTAACTGCGCTG	1204
2027	TUFB_EC_365_379_F	TGCTGATCTTCTTAAAGCGGACTTTC	284	RPOC_EC_2227_2249_R	TGCTGAGTCTTAACTGCGCTG	1244
2028	MOD_F_2146_2174_F	TGCTGATCTTCTTAAAGCGGACTTTC	617	RPOB_EC_1909_1929_TM0D_R	TGCTGAGTCTTAACTGCGCTG	1250
2029	1841_1866_F	TGCTGATCTTCTTAAAGCGGACTTTC	309	RPLB_EC_739_763_R	TGCTGAGTCTTAACTGCGCTG	1208
2030	RPLB_EC_550_579_TM0	TGCTGATCTTCTTAAAGCGGACTTTC	397	RPLB_EC_737_760_R	TGCTGAGTCTTAACTGCGCTG	1295
2031	D_F	TGCTGATCTTCTTAAAGCGGACTTTC	385	INFB_EC_1439_1469_R	TGCTGAGTCTTAACTGCGCTG	1335
2032	RPLB_EC_590_710_F	TGCTGATCTTCTTAAAGCGGACTTTC	482	VALS_EC_1195_1219_R	TGCTGAGTCTTAACTGCGCTG	1292
2033	INFB_EC_1366_1393_F	TGCTGATCTTCTTAAAGCGGACTTTC	405	SSFE_BA_197_222_TM0D_R	TGCTGAGTCTTAACTGCGCTG	1402
2034	MOD_F_1105_1124_F	TGCTGATCTTCTTAAAGCGGACTTTC	698	RPOC_EC_2313_2338_R	TGCTGAGTCTTAACTGCGCTG	1273
2035	SSFE_BA_113_137_F	TGCTGATCTTCTTAAAGCGGACTTTC	263	MRCT-R_NC003923-41798-41609_86_113_R	TGCTGAGTCTTAACTGCGCTG	1420
2056	RPOC_EC_2218_2241_F	TGCTGATCTTCTTAAAGCGGACTTTC	457	AGR-III_NC003923-2108074-2109507_36_739_R	TGCTGAGTCTTAACTGCGCTG	730
2057	MOD_F_2146_2174_F	TGCTGATCTTCTTAAAGCGGACTTTC	701	AGR-III_NC003923-2108074-2109507_622_653_R	TGCTGAGTCTTAACTGCGCTG	906
2058	MRCT-R_NC003923-41798-41609_33_60_F	TGCTGATCTTCTTAAAGCGGACTTTC	610	AGR-III_NC003923-2108074-2109507_1070_1098_R	TGCTGAGTCTTAACTGCGCTG	1319
2059	AGR-III_NC003923-2108074-	TGCTGATCTTCTTAAAGCGGACTTTC		AGR-I_AJ617706_694_726_R	TGCTGAGTCTTAACTGCGCTG	1021
2060	AGR-III_NC003923-2108074-	TGCTGATCTTCTTAAAGCGGACTTTC				

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F	F	AGR- I_AJ617706_580_611_	TGGGATTTTAAACCACTGCTGACCA TCCGAG	579	AGR-I_AJ617706_626_655_R	TGGTACTTCACTTCATCCATATGAG TC	1302
2061	AGR- II_AJ617711_250_283	AGR-II_NC002745- 2079448- 2080879_620_651_F	TCTTCGCGAGTTTATTTGATGACCC TAAAGT	415	AGR-II_NC002745-2079448- 2080879_700_731_R	TGTTTATTTGTTTCCATGATCTACACAC TTTC	1424
2062	AGR- II_AJ617711_250_283	AGR-II_NC002745- 2079448- 2080879_649_679_F	TGTATCCGCTGAATTAACGATTTAT ACGAC	624	AGR-II_NC002745-2079448- 2080879_715_745_R	TTCGACGACGTAGTTGTTATTTGTTTC CAT	1077
2063	AGR- IV_AJ617711_931_961	TGTTATCTATTTTGTCTGATATGAC CTCCG		566	AGR- IV_AJ617711_1004_1035_R	TGCTCATTCAGCATTTTCCACATATAT GTGA	1233
2064	AGR- IV_AJ617711_250_283	TGCTACTCTGCTTCTTAAATTTAGTA AACATCA		562	AGR-IV_AJ617711_309_335_R	TCCCTACTATGCGCATTAAGTTCAT TCC	1017
2065	BLAZ_NC002952(19138 27...1914672)_68_68_	TCCACTTATCCGAAATGGAAATTA GCAA		312	BLAZ_NC002952(1913827...19 14672)_68_68_R	TGGCCACTTTTATGACGACCTTCAAT C	1277
2066	BLAZ_NC002952(19138 27...1914672)_68_68_	TGCATTTATCCGAAATGGAAATTA GCAA		494	BLAZ_NC002952(1913827...19 14672)_68_68_2_R	TAGCTTTTGGACGACGCTTTTAATTA AGAT	926
2067	BLAZ_NC002952(19138 27...1914672)_68_68_	TGATCTCTCAAGCTGCTGCTTTC TAAATCA		467	BLAZ_NC002952(1913827...19 14672)_68_68_3_R	TGCGACGACGCTTTTAATTAAGATGATC CC	1263
2068	BLAZ_NC002952(19138 27...1914672)_68_68_	TATCTCTCAAGCTGCTGCTTTC TAAATCA		232	BLAZ_NC002952(1913827...19 14672)_68_68_4_R	TCTTTCTTTGCTTAATTTCCATTTGCG GAT	1145
2069	BLAZ_NC002952(19138 27...1914672)_68_68_	TGATCTCTCAAGCTGCTGCTTTC TAAATCA		487	BLAZ_NC002952(1913827...19 14672)_34_67_R	TATCTCTCAAGCTTTTATGATCTAA AGCATTA	1366
2070	BLAZ_NC002952(19138 27...1914672)_68_68_	TGATCTCTCAAGCTGCTGCTTTC TAAATCA		351	BLAZ_NC002952(1913827...19 14672)_40_68_R	TGGGACGCTTCTTACGACTTTTATGATC TAA	1289
2071	BSA-A_NC003923- 1304065-	TAGCGATGTGCTTCTTACTTCAAT T		214	BSA-A_NC003923-1304065- 1303589_253_278_R	TGACGGGACCTTAGATTTACAAACCC T	1197
2072	BSA-A_NC003923- 1304065-	ATCAATTTGTTGGCCGAGACGCGG T		32	BSA-A_NC003923-1304065- 1303589_253_278_R	TGCTATGGAAGGATTAACCATAGTT T	1203
2073	BSA-A_NC003923- 1304065-	TGATCTCTCAAGCTGCTGCTTTC TAAATCA		679	BSA-A_NC003923-1304065- 1303589_388_415_R	TAAACAGTTTACTTCCGATCCACTAA TAA	856
2074	BSA-A_NC003923- 1304065-	TGATCTCTCAAGCTGCTGCTTTC TAAATCA		519	BSA-A_NC003923-1304065- 1303589_317_344_R	TGTGTGTGCGCGATTAATTTCAATTA TAA	1353
2075	BSA-B_NC003923- 1304065-	TGATCTCTCAAGCTGCTGCTTTC TAAATCA		209	BSA-B_NC003923-1917149- 1303589_317_344_R	TGTGTGTGCGCGATTAATTTCAATTA TAA	1331

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2094	EPMB_Y13600-625- 1362_551 645 R	TRAGCTCCGCGAGATGCTTTC	161	EPMB_Y13600-625- 1362_551 645 R	TGATGTGCTATCGCGGTGATTT	989
2095	SVLUR_NCO03923- 1531285 688 713 F	TGAGCTGCATCAAGTGTATGATAG	456	PVLUR_NCO03923-1529595- 1531285 775 804 R	TGGAAATCTCATGTAATTAAGATGAAAG GA	1261
2096	PVLUR_NCO03923- 1529595- 1531285 1039 1068 F	TGGAAATTAATGCTCTCGGATTTT GACT	539	PVLUR_NCO03923-1529595- 1531285 1095 1125 R	TGATAGTGAATATGCTGGACATGTC CAA	993
2097	PVLUR_NCO03923- 1529595- 1531285 908 936 F	TGAGTACATCATATTTCTGGCATTA CCT	461	PVLUR_NCO03923-1529595- 1531285 950 978 R	TTCATGAAAAAGCTCTGGAGTACAA G	1124
2098	PVLUR_NCO03923- 1531285 610 633 F	TGCGATCTGTATGCTGCAATGCTTT	373	PVLUR_NCO03923-1529595- 1531285 654 682 R	TCCACACTGTATGAGGAAAAGGTTCA T	968
2099	SA442_NCO03923- 2538576- 2538831 11 35 P	TGTCGTGTACAGATATTTCTGACAA	635	SA442_NCO03923-2538576- 2538831 98 124 R	TTTCGATCGACCTATATGATTTCA	1433
2100	SA442_NCO03923- 2538576- 2538831 103 126 F	TGAAATCTCATATGCTTCATCGGAA A	427	SA442_NCO03923-2538576- 2538831 163 188 R	TGATATGACCACCTCTGCTACTATA	1098
2101	SA442_NCO03923- 2538576- 2538831 166 188 P	TGTCATATGCTTCTCTGGAAACA	395	SA442_NCO03923-2538576- 2538831 231 257 R	TTTATGACACGCTCTGCTACTATAA	1428
2102	SEA_NCO03923- 2052219- 2051456 115 135 P	TATACACCAAGCTGTGATACAA	226	SEA_NCO03923-2052219- 2051456 173 200 R	TGATATGAGAGGAAACTTTTTCAG	1179
2103	SEA_NCO03923- 2052219- 2051456 572 598 P	TGCTGATGATTTTTGATGCGAAG T	495	SEA_NCO03923-2052219- 2051456 621 651 R	TGATGTGACTCTCTTTATTTGATTT ATG	1070
2104	SEA_NCO03923- 2052219- 2051456 382 411 F	TGTATGTGCTGTACATTTACATAT ATATATC	156	SEA_NCO03923-2052219- 2051456 461 492 R	TGTATATATCCAGAGTCTGTGAGACT ATG	1315
2105	SEA_NCO03923- 2052219- 2051456 377 406 F	TGTATGTATGCTGTGCTTACATAC ATGAA	639	SEA_NCO03923-2052219- 2051456 455 492 R	TGACGCTTTCGAAAGTCTGTATTTTG T	861
2106	SEA_NCO02758- 2135540- 2135140 208 237 F	TTTCAATATATTTTATGATATGCA CTGA	695	SEA_NCO02758-2135540- 2135140 273 298 R	TGACGCTTTCGAAAGTCTGTATTTTG TTTACC	862
2107	SEA_NCO02758- 2135540- 2135140 206 235 F	TATTTACATATATTTTATGATATCG CACT	702	SEA_NCO02758-2135540- 2135140 402 402 R	TGATCTGTTTAGGACTCTGTGACT ATG	988
2108	SEA_NCO02758- 2135540- 2135140 206 235 F	TATTTACATATATTTTATGATATCG CACT	244	SEA_NCO02758-2135540- 2135140 402 402 R	TGCACTCATCTGTTTAGGACT ATG	1194
2109	SEA_NCO02758- 2135540- 2135140 206 235 F	TATTTACATATATTTTATGATATCG CACT	151	SEA_NCO02758-2135540- 2135140 402 402 R	TGCACTCATCTGTTTAGGACT ATG	1334

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2110	2135140_402_402_F	TGTATATATAGTGTGTATATAGTGC	596	SEQ_NC002758- 2135540- 2135140_402_402_2_R	TTACATCTTCATTAATCCGACATGAA	1361
2111	851678- 852768_546_575_F	TTCACATGTAGAGAAACACCTTTGATA ATGGA	648	SEC_NC003923- 852768_620_647_R	TGAGTGTGCACTTCATTAAGAAATGTGT	1177
2112	851678- 852768_537_566_F	TGGATATCAAAATCATGAGGAAGAAC ACTT	546	SEC_NC003923-851678- 852768_619_647_R	TCAGTGTGCACTTCATTAAGAAATGTGT	985
2113	851678- 852768_720_749_F	TGAGTGTTCACGTTCACCCATATGAAA CAGG	466	SEC_NC003923-851678- 852768_794_815_R	TGCGCTGTGTGCGCATCATAT	1078
2114	851678- 852768_787_810_F	TGATATATATATATGCTGTGCACCA	504	SEC_NC003923-851678- 852768_853_886_R	TTCTGCACCTTTTATGATATCACACGTTTT	1133
2115	851678- 852768_851_887_682_F	TGATATATATATATGATGAGACTGCTT	615	SEC_NC003923-851678- 852768_941_970_R	TGTCACCATTTATCCGAAATGTATG	1318
2116	851678- 852768_941_970_711_F	TGAGGTGTGCTGCTCCACGAA	554	SEC_NC003923-851678- 852768_1011_1040_R	TGGGACCAATTTATCCAAATATGATG	1288
2117	851678- 852768_1011_1040_F	TTTGCACACGACGAGCTATTT	683	SEC_NC003923-851678- 852768_1111_1140_R	TTGCGCTGTATTTTCTCTCCGGA	1079
2118	851678- 852768_1111_1140_F	TGAGTGTATGAGGTGATTTCCGAAA	559	SEC_NC003923-851678- 852768_1211_1240_R	TGTCAATATGAGGTGCTCTGTGATA	1320
2119	851678- 852768_1211_1240_F	TTTACATCATCTTTATATATATGCTT	699	SEC_NC003923-851678- 852768_1311_1340_R	TGATTTATTTCTGCTGTTTTCTGCTAC	994
2120	851678- 852768_1311_1340_F	TGATCATCTGCTGCTATACATTTAT	469	SEC_NC003923-851678- 852768_1411_1440_R	TGACGACCATTTATGCTCTCTCTTTTCC	870
2121	851678- 852768_1411_1440_F	TGATCATCATCTGCTATACATTTAT	445	SEC_NC003923-851678- 852768_1511_1540_R	TTCTATGATGCTAGTGTATTTGTTTTGCT	1120
2122	851678- 852768_1511_1540_F	TGATCATCATCTGCTATACATTTAT	640	SEC_NC003923-851678- 852768_1611_1640_R	TTTTCACCTTTACGCTCAAGCT	1435
2123	851678- 852768_1611_1640_F	TGATCATCATCTGCTATACATTTAT	639	SEC_NC003923-851678- 852768_1711_1740_R	TGACTTTACGCTCAAGCTCTCT	892
2124	851678- 852768_1711_1740_F	TGATCATCATCTGCTATACATTTAT	403	SEC_NC003923-851678- 852768_1811_1840_R	TTCTATGATGCTAGTGTATTTGTTTTGCT	1043
2125	851678- 852768_1811_1840_F	TGATCATCATCTGCTATACATTTAT	520	SEC_NC003923-851678- 852768_1911_1940_R	TGACTTTCTCTCTCTCTCTCTCTCTCTCT	863

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2126	SEQ_NC002758- 1955100- 1954171.633.651.F SEQ_NC002758- 1955100- 1954171.633.651.F	TGACACATGAGCAACATCTTGATTT ACA	548	SEQ_NC002758-1955100- 1954171.671.702.R SEQ_NC002758-1955100- 1954171.607.635.R	TGCTTTGTAATCTAGTTCTGTGATGTA ACCA T	1260
2127	SEQ_NC002758- 1955100- 1954171.640.564.F	TGAGGTTTGTGTATGATGTGCT	555	SEQ_NC002758-1955100- 1954171.735.762.R SEQ_NC002953-60024- 60977.547.576.R	TGCTATGTGCAATGTTTACTGTACAG T	1329
2128	SEQ_NC002758- 1955100- 1954171.654.718.F	TACAGACAGACATGCTGCTACTA	173	SEQ_NC002758-1955100- 1954171.735.762.R SEQ_NC002953-60024- 60977.547.576.R	TGATCTAATGAGCAACATGATGCTG GA	1187
2129	SEQ_NC002953-60024- 60977.449.472.F	TTCACATCTCTGATTTTACTCTAGA	662	SEQ_NC002953-60024- 60977.449.472.F SEQ_NC002953-60024- 60977.449.472.F	TAGTGTGTGACTCATATGACATCTA GA	827
2130	SEQ_NC002953-60024- 60977.408.434.F	TAGACATCAAGGTGATGATGGCAGTG	201	SEQ_NC002953-60024- 60977.408.434.F SEQ_NC002953-60024- 60977.408.434.F	TCTGAGCTTAATCTAGACATGTGCA	1390
2131	SEQ_NC002953-60024- 60977.408.434.F	TACTGATCTCTATAGAGGTACACAC ACTA	400	SEQ_NC002953-60024- 60977.408.434.F SEQ_NC002953-60024- 60977.408.434.F	TACCATCTACCCCAACATATAGACCAA	888
2132	SEQ_NC002953-60024- 60977.546.575.F	TTCGTGATCTCTATGATGGAGTACAA CACT	677	SEQ_NC002953-60024- 60977.594.616.R SEQ_NC002758-1957830- 1956949.420.447.R	TAGCAACATCACTCTTCTCTGT	909
2133	SEQ_NC002758- 1957830- 1956949.324.349.F	TGACTCTGATTTTCAACAGGTACCA	253	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TGACAGACATCAATATCATCTGCCAA	966
2134	SEQ_NC002758- 1957830- 1956949.336.363.F	TTCACAGGTACCAATGATTTGATCT CA	666	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TGTACAGACATCAATATCATCTGCCA	1316
2135	SEQ_NC002758- 1957830- 1956949.356.384.F	TGATCTCAATCTATATATCTGGC GAA	471	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TCTGGCCCTCCCAACATGATTTAG	1129
2136	SEQ_NC002758- 1957830- 1956949.223.253.F	TCTCAAGGTGATTTGGGTAGGTAA CTTAA	394	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TGGTAGTGTTTTATCTGTGACCTCTT	1293
2137	SEQ_NC002758- 1957830- 1956949.223.253.F	TGTGAGGTATCACTCTCATGAACAA	637	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TCTAGCGAACACATCTCTGATG	1119
2138	SEQ_NC002758- 1957830- 1956949.223.253.F	TGATCTCAATCTATATATCTGGC GAA	211	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TCTAGCGAACATCTCTGATGATG	1049
2139	SEQ_NC002758- 1957830- 1956949.223.253.F	TGATCTCAATCTATATATCTGGC GAA	153	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TGATCTCTCTGATTTTATCTGATG	925
2140	SEQ_NC002758- 1957830- 1956949.223.253.F	TGATCTCAATCTATATATCTGGC GAA	301	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TCTAGCGAACACATCTCTGATG	984
2141	SEQ_NC002758- 1957830- 1956949.223.253.F	TGATCTCAATCTATATATCTGGC GAA	619	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TCTAGCGAACACATCTCTGATG	1312
2142	SEQ_NC002758- 1957830- 1956949.223.253.F	TGATCTCAATCTATATATCTGGC GAA	514	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TCTAGCGAACACATCTCTGATG	1221
2143	SEQ_NC002758- 1957830- 1956949.223.253.F	TGATCTCAATCTATATATCTGGC GAA	304	SEQ_NC002758-1957830- 1956949.420.447.R SEQ_NC002758-1957830- 1956949.420.447.R	TCTAGCGAACACATCTCTGATG	907

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2144	TSST_NCO02758- 2138255 397 325 P 2138255 347 373 R	TCCTTTTCCAAAGGGGAAAAGTTGA CTT	423	TSST_NCO02758-2137564- 2138255 347 373 R	TAACTCTTCTCCATGATGATGCTT	974
2145	ARCC_NCO03923- 2725050- 2724595 37 58 F 2724595 37 120 R	TSCCGGCAATCCCACTGATGATA ATCGT	368	ARCC_NCO03923-2725050- 2724595 37 120 R	TCGATTAATTCACATTCATTTCTGATTT	1175
2146	ARCC_NCO03923- 2725050- 2724595 131 161 F 2724595 218 249 F	TGAATATGCTAAGAACTGTAGAGCA ATCGT	437	ARCC_NCO03923-2725050- 2724595 218 249 R	TCCTCTCTTCTGATAAAGAGCA TTGG	1137
2147	ARCC_NCO03923- 2725050- 2724595 171 393 P 2724595 322 353 R	TTGGTCTCTTTTATACGAAGAGAA GTTGAA	691	ARCC_NCO03923-2725050- 2724595 322 353 R	TGGTGTCTGATGATGATGAGTAGTG GTGA	1306
2148	ARCC_NCO03923- 1674726- 1674277 30 62 F 1674277 155 181 R	TTGCGATGAGAGATGCTGCTGT ATTCTCA	686	ARCC_NCO03923-1674726- 1674277 155 181 R	TGAAATGCTGATTAATCTTCTGACAT CT	1064
2149	ARCC_NCO03923- 1674726- 1674277 204 232 F 1674277 308 335 R	TGGGCTTTAAATATCCAAATGAAG ATTCTCA	590	ARCC_NCO03923-1674726- 1674277 308 335 R	TACTGCTAATCTCTCTGTCATCA	891
2150	GLFP_NCO03923- 1296927- 1297391 270 301 F 1297391 382 414 R	TGATGGCAATGAGATAGGATATAA CAG	474	ARCC_NCO03923-1296927- 1297391 382 414 R	TAAACAATCTCTTACTTGCACACCTG	869
2151	GLFP_NCO03923- 1296927- 1297391 27 51 F 1297391 81 100 R	TGCAACCGATTAAGAAATCATTTTG CGAAT	491	GLFP_NCO03923-1296927- 1297391 81 100 R	TGCAACATTAATGCTCCGACAAATAAA GAAAT	1193
2152	GLFP_NCO03923- 1296927- 1297391 239 260 F 1297391 323 359 R	TGATGGGATAGAGGATTAAGATG TAGCTGACCGAATTAAGTGT	558	GLFP_NCO03923-1296927- 1297391 323 359 R	TAAAGACCGCTGGTGTAAATGCA	850
2153	OMK_NCO01923- 1191334 91 122 F 1191334 166 197 R	TACTCTTAAACTAGGATGCTGCTT TGACG	218	OMK_NCO01923-1190906- 1191334 166 197 R	TACCAATTAATAAATTAATCACTTAAGTTA ATGCAATG	972
2154	GLFP_NCO03923- 1190906- 1191334 240 267 F 1191334 403 432 R	TGAATGAGAGATGCAAGCAATTA CA	200	OMK_NCO03923-1190906- 1191334 403 432 R	TGAATTAATGATGCTGATCAATTAATG TGCC	1180
2155	OMK_NCO03923- 1190906- 1191334 301 329 P 1191334 403 432 R	TGATGAGAGATGCAAGCAATTA CA	435	OMK_NCO03923-1190906- 1191334 403 432 R	TGCTCTCTGAGATGCTTAAGTTGA G	1082
2156	PTA_NCO03923- 623885- 623935 237 263 F 623935 314 345 R	TGCTCTCTGAGATGCAAGCAATTA CA	268	PTA_NCO03923-623885- 623935 314 345 R	TGCGAGCTAATGCTAATAATTAATTAAT TC	1284
2157	PTA_NCO03923- 623885- 623935 141 171 F 623935 211 239 R	TGCTCTCTGAGATGCAAGCAATTA CA	418	PTA_NCO03923-623885- 623935 211 239 R	TGCTCTCTGAGATGCTTAAGTTGA G	1301
2158		TGAATTAATGATGCTAATAATTAATTAAT GAGCT	439		TGCTCTCTGAGATGCTTAAGTTGA G	1207

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2159	PTA_NCO03923- 629555 328 356 F	TCCBACCGGGGTATCAGACATC AGG	303	PTA_NCO03923-629885- 629555 393 422 R	TGTCTGGATTGATGACACATCAGCA AG	1349
2160	TFI_NCO03923- 830671- 831072 131 160 F	TGAGATTAGAAAGCTGTGCAAGT TTAT	486	TFI_NCO03923-830671- 831072 209 239 R	TGAGATTGATGATTTACCACTTCCGA TTG	1165
2161	TFI_NCO03923- 831072 1 34 F	TCCACGACAGATGAGAAATTA CAAAAAG	318	TFI_NCO03923-830671- 831072 97 129 R	TGGTCAACATCTGATGCTTACCACTT TACG	1300
2162	TFI_NCO03923- 830671- 831072 199 227 F	TCCAACTGGGCAATCGGAATGATTA ATC	246	TFI_NCO03923-830671- 831072 293 286 R	TGGCAGATGATGACGTGACAAATGC ACACAT	1275
2163	VOI_NCO03923- 379431 142 167 F	TGATTCCTGCTGATGAAAGTGGCTT T	440	VOI_NCO03923-378916- 379431 259 284 R	TGCCAGCTAGACAGATGCAATTC T	1076
2164	VOI_NCO03923- 379431 44 77 F	TACACATATATTAAGAAAGCGGT TTGATTC	175	VOI_NCO03923-378916- 379431 120 145 R	TTGCTGCTGGATTTGCTGCTGCT T	1388
2165	VOI_NCO03923- 378916- 379431 135 160 F	TCCACGACGATTTGCTGCTGATGAG T	314	VOI_NCO03923-378916- 379431 193 221 R	TCCAAACCGACACGACATCTATATCA C	987
2166	VOI_NCO03923- 378916- 379431 235 300 F	TGCTGGCGGTGAGGAAATATGCT T	219	VOI_NCO03923-378916- 379431 364 396 R	TCCACGATTTAACCTGCTATACCAAG CTATC	1013
2167	BLAZ_NCO03923- 1914672 546 575 F	TGCACTCTCGAAATGGAATTA GCA	312	BLAZ_NCO03923-1914672- 55 653 R	TGGCCACTTTATCAGACACCTTAGCT C	1277
2168	BLAZ_NCO03923- 1914672 546 575 2 F	TGCACTATGCAAAATGGAATTA GCA	494	BLAZ_NCO03923-1914672- 28 659 R	TAGTCTTTTGGACACGCTTTATTA AAGT	926
2169	BLAZ_NCO03923- 1914672 507 531 F	TGATCTCTGACGCTCTGCTTTC T	467	BLAZ_NCO03923-1914672- 22 651 R	TGGACACGCTCTTTATTAAGATATCT CC	1263
2170	BLAZ_NCO03923- 1914672 508 531 F	TGATCTCTGACGCTCTGCTTTC T	232	BLAZ_NCO03923-1914672- 53 593 R	TCTTTCTCTTCTTAAATTTGATTTGC C	1145
2171	BLAZ_NCO03923- 1914672 546 575 F	TGCACTCTCTAGTTTATGATGCT GTGATTC	487	BLAZ_NCO03923-1914672- 21 651 R	GATTTCTCTTACCACTTTTATGATTA AATTA	1366
2172	BLAZ_NCO03923- 1914672 546 575 2 F	TGCTGCTCTTATGTTTATGACATG AATTA	351	BLAZ_NCO03923-1914672- 27 157 R	TGGGACATCTCTTACCACTTTATGATC TAA	1289
2173	BLAZ_NCO02952- 1914672 546 575 F	TGCACTATCGAAATGGAATTA GCA	312	BLAZ_NCO02952-1913827- 1914672 655 683 R	TGCCACCTTTATGCAACCTTACATG C	1277
2174	BLAZ_NCO02952- 1913827- 1914672 546 575 2 F	TGCACTATCGAAATGGAATTA GCA	494	BLAZ_NCO02952-1913827- 1914672 628 659 R	TAGTCTTTTGGACACGCTTTATTA AAGT	926
2175	BLAZ_NCO02952- 1913827- 1914672 507 531 F	TGATGCTTACGCTCTGCTTTC T	467	BLAZ_NCO02952-1913827- 1914672 622 651 R	TGGACACGCTCTTTATTAAGATATCT CC	1263

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2176	BLAZ NC002352- 1914827- 1914827-508 531 F	TAATCTTCAGCGCTGCTGCTTC	232	BLAZ NC002352-1913827- 1914672 553 583 R	TCATTCCTTGCTTAATTCCTCATTCG	1145
2177	BLAZ NC002352- 1913827- 34 56 F	TCGAACTGCTTTAGTTTATAGTCGAT	487	BLAZ NC002352-1913827- 1914672 121 154 R	TTACTCTTCACCTCTTTAGTATCTAA AGCTA	1366
2178	BLAZ NC002352- 1913827- 26 58 F	TCGCTGGCTTAGTTTATAGTCGATG AATTCGA	351	BLAZ NC002352-1913827- 1914672 127 157 R	TGGGACATCTCTACCACTTTAGTATC TAA	1289
2247	TUPE NC002758- 615038- 616222 693 721 F	TGTTGACCTGGTCAATCAAAAGTGG GTG	643	TUPE NC002758-615038- 616222 175 259 R	TTGTCACGACTGAGGTAGTCTAATA	1321
2248	TUPE NC002758- 615038- 616222 696 716 F	TGCTGTGACAGTGGTGTATATCAAG T	386	TUPE NC002758-615038- 616222 793 820 R	TTGTCACGACTGAGGTAGTCTAATA	1321
2249	TUPE NC002758- 615038- 696 725 F	TGACCTGGTGTCAATCAAGTTGGTG AAGA	430	TUPE NC002758-615038- 616222 793 820 R	TTGTCACGACTGAGGTAGTCTAATA	1321
2250	TUPE NC002758- 615038- 616222 488 513 F	TGCGAGCTGGACATTTACTCTATATC	320	TUPE NC002758-615038- 616222 601 630 R	TGGTTCCTGACATCACTGCTTGGATTT GG	1311
2251	TUPE NC002758- 615038- 616222 945 972 F	TGAGGTGAGAGTCACTCACTCCATTC TC	433	TUPE NC002758-615038- 616222 1030 1069 R	TAGCGATACACTTCATGATCACTTCGG TAA	922
2252	TUPE NC002758- 616222 333 356 F	TCGATGTCGCAAACTGTGAGCA	307	TUPE NC002758-615038- 616222 424 459 R	TTCCATTCCTCACTTAATTCATTAATCT TCAATGTC	1382
2253	NUC NC002758- 894288- 894288-002 434 F	TCCTGATGCACTGATCAATTAAGA	342	NUC NC002758-894288- 894374 165 189 R	TACGTATACCGCTTCGATATATATCA	899
2254	NUC NC002758- 894288- 894574 53 81 F	TCCTTATGAGATGATGATCACTGAT GTT	349	NUC NC002758-894288- 894574 222 250 R	TGTTTGTGATGCTTTGCTGAGCTA	1354
2255	NUC NC002758- 894288- 894574 169 194 F	TGCGAATATGCTATCAACAGATATA	273	NUC NC002758-894288- 894574 222 250 R	TAGTGTGAGTTGCTATATATATATG A	928
2256	NUC NC002758- 894288- 894374 316 345 F	TCAAGATGTACCAATGATCACTTCAG ACTA	174	NUC NC002758-894288- 894574 396 421 R	TAAATGATCTGCTTCGCGGCGATAT	853
2270	RPOB EC_3796 3821 F	TGAGAGTGGGTGGGTGATATGTA	566	RPOB EC 3868 3895 R	TCAGCTGTGCACTTCACGTGAGAT TGGTGGGACTTAAAGGTGCGAGTTCTT GCA	979
2271	RPOB EC_3789 3812 F	TGAGTGGGAGTGTGATGCTTCGG	294	RPOB EC 3860 3890 R	TGGTGGGACTTAAAGGTGCGAGTTCTT GCA	1107
2272	RPOB EC_3789 3812 F	TCAGTGTGGGCTGTCGCTTCGG	294	RPOB EC 3860 3890 2 R	TGGTGGGACTTAAAGGTGCGAGTTCTT GCA	1102

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2327	3772_1555_1581_P INV 122457-74- 3772_1558_1585_P	A TGGTAAACAGAGGCTTACAGGCGATA TG	598	3772_1619_1647_R INV 122457-74- 3772_1622_1652_R	A TACATAGAGGTTGCGTTCGACGATATCTTT TAC	987
2328	ASD NC006570- 439714- 439608_3_37_F	TGAGCGTTTATCTTAAAGTGGTT TTATATGGTT	459	ASD NC006570-439714- 439608_51_84_R	TGATTCGATACACAGAGCATATAAAGCT GAG	1188
2329	ASD NC006570- 439714- 439608_18_45_F	TAAAGTGGTTTATATGGTTGCGCG GA	149	ASD NC006570-439714- 439608_66_95_R	TCGAATCTTTTGATCTCATACAGAG AC	948
2330	ASD NC006570- 439714- 439608_17_45_F	TTAAAGTGGTTTATATGGTTGCGCG GGA	647	ASD NC006570-439714- 439608_67_95_R	TCGCAATCTTTGATCTCATACAGAG TCC	1016
2331	ASD NC006570- 439714- 439608_9_40_F	TGTATGCTTAAAGTGGTTTATATG GTGCG	709	ASD NC006570-439714- 439608_107_114_R	TCGCTGCGAGATGTCGAAAGACGTTG ATG	1128
2332	GALE AF513299_171_2 00_F	TGAGCTAGACCTTTAGATGAAGCTA ACCT	280	GALE AF513299_241_271_R	TACGATGTAAATTCGCAAGACTTTG GCTTTAG	900
2333	GALE AF513299_168_1 99_F	TTATCACTAGACCTTTAGGTTAAG CTAAGC	658	GALE AF513299_245_271_R	TCGCACTACAGCTTTAAGCCGCGA TACAGCTTTAAGAGCGCGAATGATTT	1121
2334	GALE AF513299_168_1 99_F	TGCACTAGACCTTTAGGTTAAG CTAAGC	658	GALE AF513299_233_264_R	TCGCACTACAGCTTTAAGCCGCGA TACAGCTTTAAGAGCGCGAATGATTT	883
2335	GALE AF513299_169_1 99_F	TGCACTAGACCTTTAGGTTAAG CTAAGC	319	GALE AF513299_252_279_R	TCGCACTACAGCTTTAAGCCGCGA TACAGCTTTAAGAGCGCGAATGATTT	1374
2336	PIA AF053945_7377_7 403_F	TTGAGAGAGATCCGCGTCACTAT TGTGGA	680	PIA AF053945_7434_7468_R	TGATTCGCGAGAGATTTGCGATTAG TGT	1035
2337	PIA AF053945_7377_7 403_F	TGCACTCGGCTCACTATTAAGTT AC	443	PIA AF053945_7428_7455_R	TGATTCGCGAGAGATTTGCGATTAG TGT	854
2338	CAE AF053947_33412_1 33441_F	TCGTTATCGCATGTCATATTGCG AACT	444	CAE AF053947_33412_1 33441_F	TGATTCGCGAGAGATTTGCGATTAG TGT	866
2339	CAE AF053947_33412_1 33458_F	TGCTTATTCGCACTATTCGCACTG CTAATGC	329	CAE AF053947_33483_33507_1 R	TGATTCGCGAGAGATTTGCGATTAG TGT	1308
2340	CAE AF053947_33407_1 33429_F	TCGTTATTCGCACTATTCGCACTG CTAATGC	499	CAE AF053947_33483_33507_1 R	TCGCACTACAGCTTTGCGATTAG TGT	1373
2341	CAE AF053947_33407_1 33429_F	TCGTTATTCGCACTATTCGCACTG CTAATGC	291	CAE AF053947_33483_33507_1 R	TGATTCGCGAGAGATTTGCGATTAG TGT	1184
2342	CAE AF053947_33407_1 33429_F	TCGTTATTCGCACTATTCGCACTG CTAATGC	293	CAE AF053947_33483_33507_1 R	TGATTCGCGAGAGATTTGCGATTAG TGT	1060
2343	CAE AF053947_33407_1 33429_F	TCGTTATTCGCACTATTCGCACTG CTAATGC	260	CAE AF053947_33483_33507_1 R	TGATTCGCGAGAGATTTGCGATTAG TGT	967
2344	CAE AF053947_33407_1 33429_F	TCGTTATTCGCACTATTCGCACTG CTAATGC	507	CAE AF053947_33483_33507_1 R	TGATTCGCGAGAGATTTGCGATTAG TGT	947
2472	OMPA NC000117_68_89 71_F	TGCTGTTAGGATCTCTCTGA	475	OMPA NC000117_757_777_R	TGCTGTTAGGATCTCTCTCTGA	1328
2473	OMPA NC000117_798_8 71_F	TGCTGTTAGGATCTCTCTCTGA	475	OMPA NC000117_757_777_R	TGCTGTTAGGATCTCTCTCTGA	1328
2474	OMPA NC000117_645_6 71_F	TGCTGTTAGGATCTCTCTCTGA	521	OMPA NC000117_757_777_R	TGCTGTTAGGATCTCTCTCTGA	1328

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2475	OWPA_N0000117_947_9 73 F	TACGTGATGAGACACCTCTTACTGA	157	OWPA_N0000117_1011_040_R AG	TGACGAGCAGCACTCTGAGAGTCTG AG	1153
2476	OWPA_N0000117_774_7 2476	TACTGAGACAGAGCTGAGACC	196	OWPA_N0000117_871_894_R	TGAGAGAGTTGCTGAGAGCACTG	1371
2477	OWPA_N0000117_457_4 83 F	TCTCTCTGCTTGCTGTATTCGAGGT T	676	OWPA_N0000117_511_534_R	TAAAGAGAGCTTGCTGTAGTCTCATTCG GA	851
2478	OWPA_N0000117_687_7 10 F	TAGCCGAGCAGCAATTTGATGATCA C	212	OWPA_N0000117_787_815_R	TGACCATCTCACTGGTATTTAAGTGTAGCA	1406
2479	OWPA_N0000117_540_5 56 F	TGCGGTAGTAGACATTTATTCAGACA	571	OWPA_N0000117_649_672_R	TCTCTGAGACGAGGTTTGATATG	1395
2480	OWPA_N0000117_338_3 50 F	TGCGCATTCGCGATATGTTGCTCA	492	OWPA_N0000117_417_444_R	TGCTTTAAATATACGCTTAGTACTCTT	1058
2481	OWPA_N0000117_19_40 F	TATGACCAACTCATCATGAGCAG	234	OWPA_N0000117_71_91_R	TCCGCTGCGGAAATATACCTG	1025
2482	OWPA_N0000117_354_3 82 F	TGCTGAGGTGAGGATCTCTTATCTCA TTG	516	OWPA_N0000117_445_471_R	TGATCTCATCTGCTTACGAACTCAGCTTC	1270
2483	OWPA_N0000117_1297_1 1319 F	TGGAAGAGGTGTGAGCTACTCTCA	537	OWPA_N0000117_1396_1419_R	TACGTTTGCTCTCTCTGAGAAC	903
2484	OWPA_N0000117_1465_1 1453 F	TCTGCTGCAACAGAGAGAGATATAC AGG	407	OWPA_N0000117_1541_1569_R	TGCTTTCAAGTTGACAGAAACTCTACA G	1052
2485	OWPA_N0000117_44_66 F	TGACGATCTTCGCGTGAATGT	450	OWPA_N0000117_120_148_R	TGTGAGCTAGCTATTAAGTTTGTAGA G	1323
2486	OWPA_N0000117_166_1 90 F	TGACGAGCAGAGAGGTTAGACTGTC C	441	OWPA_N0000117_240_261_R	TGACATGCTTCCTCTTCTCAAG	1396
2487	OWPA_N0000117_514_5 36 F	TGAGCATTTGCGTGTGAGATGCG	287	OWPA_N0000117_640_660_R	TGCTGTGAGGAATCTAGGAGCC	1251
2488	OWPA_N0000117_801_8 27 F	TGTGATATATCTAGATGATGATGAGC A	636	OWPA_N0000117_871_893_R	TGCTGAGACTCTCTGCGAGACTC	1419
2489	OWPA_N0000117_219_2 42 F	TGTATGATGATATATCACTCTCA	632	OWPA_N0000117_319_345_R	TGATCTGATGAGCAAGTGTACTCTG	1010
2490	OWPA_N0000117_964_9 83 F	TATGAGCATCTCCCACTCTCA	176	OWPA_N0000117_1024_1041_R	TGCGAGCTGCTGCTGCGAC	1073
2491	OWPA_N0000117_1505_1 1520 F	TGCGCCGCGAGAGCT	366	OWPA_N0000117_1546_1562_R	TGCTGCTGCTTGGGATTA	1416
2492	OWPA_N0000117_216_2 39 F	TGAGCTCATCTCATCTGATGATG	279	OWPA_N0000117_124_143_R	TGCGATGCACTGCGCTGAG	1279
2493	OWPA_N0000117_219_2 42 F	TGAGTGTGATGATGATGATGATGAGC C	452	OWPA_N0000117_313_333_R	TGCGAATGCTGCTGCTGCTGCTG	1032
2494	OWPA_N0000117_115_1 41 F	TGAGTCTGATGATGATGATGATGATG C	625	OWPA_N0000117_308_330_R	TGAGTCTGCTGCGCTGCAACA	873
2495	OWPA_N0000117_517_5 39 F	TGAGCATGCTGCTGATGATGATGATG C	453	OWPA_N0000117_220_242_R	TGCGGTGATCTACTTACGAGTAC	1296
2496	OWPA_N0000117_273_2 F	TGAGCATGCTGCTGATGATGATGATG C	287	OWPA_N0000117_643_663_R	TGCTGTGAGGGAATCTAGGAGCC	1251
2497	OWPA_N0000117_273_2 F	TGAGCATGCTGCTGATGATGATGATG C	380	OWPA_N0000117_338_360_R	TGCGGATCTACTTACGAGTAC	1296

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93. F	GYFA NC000912_257_2	TSAGTAAGTTCACCCACCACG	462	GYFA NC000912_346_370 R	TGAGCGCAGGTTACCTGCTGCTC	1067
2498	ABCC NC003923- 2725050- 2724595_135_161P F	TAGTGATATGAGAACTGTAGCGAC PAACTCGCT	229	ABCC NC003923-2725050- 2724595_214_239P R	TCTGTTTCCTGATATAAAGACGCGA TATTTGCG	1116
2504	PTA NC003923- 628885- 629355_237_263P F	TCTTGTPTTGTATGCGTPTGATAAGC AGATGCG	417	PTA NC003923-628885- 629355_314_342P R	TACACGCTGTGTTTGTGCTGTGTGT TGATATATTTGTGTGTGCTTACTACAA TGAGC	904
2505	CMJLST_STL_1852_188	TTTCGCGATGAAGTGTGCGTCACTCT TTTTCG	708	CMJLST_STL_1945_1977 R	TCGCTCTCCGCGAAGACAAATGA CTACACACTCTGTTTATTTGCTTCTT	1355
2517	CMJLST_STL_2363_239	TGAGATGTGCTACAGCCCTTTAGAC ACGCG	428	CMJLST_STL_3073_3097 R	TCGCTCTCTCCGCGAAGACAAATGA CTACACACTCTGTTTATTTGCTTCTT	1020
2518	CMJLST_STL_2350_237	TTTGTGTGATGTGATGCGAGTGT TGG	535	CMJLST_STL_2447_2481 R	TGCGAAACAGAGATGATTTCTGCTGC CNA	1117
2519	CMJLST_STL_654_684	TATTCGAGAACGCTGACAAATAA GCGAT	240	CMJLST_STL_725_756 R	TCGTATGCTACAGCTGTTCGAAAC ATATG	1084
2520	CMJLST_STL_360_395	TCGCTGTATTTCTGAGCTAGTGAATC AAGTTGTGA	347	CMJLST_STL_454_487 R	TTTATGCTACTTCTAGCTGCCTTCC A	1245
2521	CMJLST_STL_1231_125	TGCGAGTTTATCAGAGCTGCTGTTCA TC	564	CMJLST_STL_1312_1340 R	TCGAGAACGACGACCTATTTCATCTT TA	1427
2522	CMJLST_STL_3543_357	TGCTGTAGCTTATCCGCGAATGTCTT TGATTT	529	CMJLST_STL_3656_3685 R	TGTTCCATACAGCTGTCCGCGAATG AT	950
2523	CMJLST_STL_1312_134	TAGAGCTTTCCGCTATGATGCGGTG AAGATAT	145	CMJLST_STL_55_84 R	TTTCGCGACTTAATTTGTGATAGCA TAGGGA	1348
2524	CMJLST_STL_1312_134	TAGAGCTTTCCGCTATGATGCGGTG AAGATAT	538	CMJLST_STL_1283_1417 R	TCGAAACGATCTACACCTACAAAG CGTTTGA	1432
2525	CMJLST_STL_254_228	TGCGCTTAATGCGCTTAATGCTAG AAGATG	582	CMJLST_STL_2352_2379 R	TGCGAAACGATCTACACCTACAAAG CGTTTGA	996
2526	CMJLST_STL_1380_141	TGCTTCTCTAGGCTTATCCAAATTT AGATGCG	534	CMJLST_STL_1486_1520 R	TGCTGTCTGATGCTATGATCAATTA ABAC	1205
2527	CMJLST_STL_3413_343	TTGTATATCCGCTGCTCTCGATGCT A	692	CMJLST_STL_3511_3542 R	TGATGATGCTATGCGGAGGATGCT TAGCTATCTCTCTAGATGTGAAT TTGCG	1257
2528	CMJLST_STL_1130_115	TACGCTGCTAAGCGCTTTCGTATG A	169	CMJLST_STL_1203_1230 R	TGATGATGCTATGCGGAGGATGCT TAGCTATCTCTCTAGATGTGAAT TTGCG	920
2529	CMJLST_STL_2840_287	TGCGGCTTCTGCTTTATGTTTITAC ATTATGAG	591	CMJLST_STL_2940_2973 R	TGATGATGCTATGCGGAGGATGCT TAGCTATCTCTCTAGATGTGAAT TTGCG	917
2530	CMJLST_STL_2058_208	TATGATGAGTGTGCTTTATGATGATG A	241	CMJLST_STL_2131_2162 R	TGATGATGCTATGCGGAGGATGCT TAGCTATCTCTCTAGATGTGAAT TTGCG	1417
2531	CMJLST_STL_553_585	TGCTGATGCTGATGCTGCTTTTGA ATGCTTT	344	CMJLST_STL_655_695 R	TGATGATGCTATGCGGAGGATGCT TAGCTATCTCTCTAGATGTGAAT TTGCG	942
2532	GYFA NC002163- 1604930- 1604529_302_338 F	TGATGTGAGCTTAACCTCTATAGAG TAAGAGC	299	GYFA NC002163-1604930- 1604529_352_380 R	TTTGTCTGATGCTCTGATGAGTAA A	1443
2544	UNCA NC002163- 1604529_302_338 F	TCGCCAGCTCTTAATTTGATGATG TAAGAGC	322	UNCA NC002163-112166- 1604529_352_380 R	TGATGATGCTATGCGGAGGATGCT TAGCTATCTCTCTAGATGTGAAT TTGCG	1065
2555	UNCA NC002163- 1604529_302_338 F	TCGCCAGCTCTTAATTTGATGATG TAAGAGC	322	UNCA NC002163-112166- 1604529_352_380 R	TGATGATGCTATGCGGAGGATGCT TAGCTATCTCTCTAGATGTGAAT TTGCG	1065

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	112166- 112647_40_113_F	GATTGAG		112647_446_171_R	
2566	UNCA_NCO02163- 112166- 112647_233_259_F	TAAATGATGATGAGTGGGGTCTT T	170	UNCA_NCO02163-112166- 112647_234_329_R	TGGATTAACATCTGTTGGATTAATACCA GAAACATC
2567	FGN_NCO02163- 327773- 328270_272_305_F	TCCTGAGTACTTAAATGAGGAGATA AAATATTT	414	FGN_NCO02163-327773- 328270_365_396_R	TGCATCCCACTTTTTCACATACGCTTA AANA
2568	FGN_NCO02163- 1569415- 1569873_285_284_F	TATGAGAGCTGTCTTTAGCGAC TTCA	661	TKT_NCO02163-1569415- 1569873_350_383_R	TGAAAGAGCAATTTTACATCTCTGTAA AGCTTA
2570	UNCA_NCO02163- 1604529_39_68_F	TGCTCTTTGATATTTTCTCTGATA ATGC	381	UNCA_NCO02163-1604529- 1604529_109_142_R	TGTCATGCTTTAAATGATCAGGATPAAA AGCACT
2571	TKT_NCO02163- 1569415- 1569903_33_62_F	TGATCTTAAATTTTCGCGCACTTC ATTC	472	TKT_NCO02163-1569415- 1569903_139_162_R	TGCTATGACAGACCTTACAGCAAT TACATCTCTTCGTAGAAATTTCTATG
2572	TKT_NCO02163- 1569415- 1569903_207_239_F	TGAGTTTATGCTTTGTGAGATG GGGATTT	164	TKT_NCO02163-1569415- 1569903_313_345_R	TGACAGAGGTTTGTGATTTTATGAC TTGTT
2573	TKT_NCO02163- 1569415- 1569903_350_383_F	TAGCTTTACAGAAATGTAAATAG CGTTTGA	213	TKT_NCO02163-1569415- 1569903_449_481_R	TGCCATGACAGGCTTAGGCAAT TTCCATGACAGGCTTAGGCAAT
2574	UNCA_NCO02163- 1569415- 1569903_60_92_F	TGAAATCTCCAGGCGCTCTGAAA TTTCAC	665	UNCA_NCO02163-1569415- 1569903_139_162_R	TGCATTTCCGATGATCTTCTCTCAACA TT
2575	UNCA_NCO02163- 1504930- 1504529_39_70_F	TGCTCTTTTGAATCTTCTCTGATA ATGCTC	382	UNCA_NCO02163-1504930- 1504529_139_162_R	ATGCTCTTACTTGGCTTACGCTAAAT TTCCA
2576	UNCA_NCO02163- 367572- 368079_386_414_F	TCACTATTCTTCAGGATATCCAGG TGG	281	UNCA_NCO02163-367572- 368079_476_508_R	TGTCACCTGCTACACATCTCCAGCA TGT
2577	UNCA_NCO02163- 367572- 368079_246_174_F	TGGTGAGATGCTTATGCTCTATTA T	611	UNCA_NCO02163-367572- 368079_242_270_R	TGTCATCTGATACCTCGGAAATATAC TGAAT
2578	UNCA_NCO02163- 367572- 368079_238_327_F	TGAGTCTCTCAGCCCAAGACT TACG	622	UNCA_NCO02163-367572- 368079_381_416_R	TGACCTCTACACATTAATAAGCTCT CA
2579	UNCA_NCO02163- 367572- 368079_1_27_F	TGAGGCAATTAACATGATGCA A	614	UNCA_NCO02163-367572- 368079_52_81_R	TGTCATCTGATACCTCGGAAATATAC TGAAT
2580	UNCA_NCO02163- 327746- 328270_254_285_F	TAGCACTGGGCTTTGAAAGATTT TTNAT	455	UNCA_NCO02163-327746- 328270_356_379_R	TTTTCTCTCGCGAAATTTTCAC TTTCTCTCTCATGATAGTACGATC
2581	UNCA_NCO02163- 327746- 328270_241_267_R	TGAAGAGTGAAGTACCAATGAG ATGAG	425	UNCA_NCO02163-327746- 328270_241_267_R	TTTTCTCTCGCGAAATTTTCAC TTTCTCTCTCATGATAGTACGATC

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2582	328270_13_182_F PBM_NC002163- 327746- 328270_19_50_F	TGCGCTTAATGCGCTTAATCAATGA AAATATG	568	PBM_NC002163-327746- 328270_19_102_R	TGCGCAAGAGCTTACTCAGC	1200
2583	UNCA_NC002163- 112647_114_141_F	TBAGCTGCTGCTGCTATGCGA TG	160	UNCA_NC002163-112166- 112647_196_225_R	TGCGCTTCTAAGAGCTCTGAGTGAGA TA	1220
2584	UNCA_NC002163- 112166- 112647_3_25_F	TGCTGCGACTCAGCAGCTCAT A	532	UNCA_NC002163-112166- 112647_88_123_R	TGCTGCTCTCACTCAATCATCATCAACA ATTAAAGC	1206
2585	ASPA_NC002163- 96692- 97166_304_335_F	TTATTTTGCCTAAATGCAACAGST AG	652	ASPA_NC002163-96692- 97166_403_432_R	TGCAAAATGAGCGTTACATCTGCTCA AT	1192
2586	GLNA_NC002163- 96692-228_258_F	TGCGTTGCGACMAACTTCTAAG TATGT	370	ASPA_NC002163-96692- 97166_316_346_R	TGATGATGAGACTACTGCTGCTGCTTT TGG	991
2587	GLNA_NC002163- 96692-244_275_F	TGCGATCATGCTAAGAGTTTCCGA TAGCTA	547	GLNA_NC002163-658085- 957609_340_371_R	TGAGTTTGACCATTTCCAGAGCAAT CTAC	1176
2588	TCT_NC002163- 1569415- 1569903_107_130_F	TGCGTACAGCGCCTTAGGCAAG	371	TCT_NC002163-1569415- 1569903_212_236_R	TGCGCATTTCCGCAAGCAATAGA TCC	1020
2589	GLNA_NC002163- 367572- 368095_214_246_F	TGTTTCTTTAGCAGACTTCCAAACT TGATTA	642	TCT_NC002163-1569415- 1569903_361_393_R	TGCTTGCTCTGAAAAGCATTTTACA TTTC	1057
2590	GLNA_NC002163- 367572-214_246_F	TGCTACTCTTTTGTGATATGAC ATATTC	505	GLNA_NC002163-367572- 368095_317_340_R	TGCTTGGGCGCGAAGTTTT	1047
2591	GLNA_NC002163- 367572-415_444_F	TGCTTTGATGCTATGATATGCTGA DAGC	353	GLNA_NC002163-367572- 368095_485_516_R	TGCTGAGCATGCTGCTACTTGTGTTG CMTA	1141
2592	P_NC002163_21_54_F	TGCTAAGCACTATATCAATGA ATATGGA	332	P_NC002163_116_142_R	TGCAAGATCCGCTCATGCTCAAG AAT	949
2593	GLNA_NC002163- 658085- 657609_148_179_F	TGATGAGAAAGCGAAGTGGCTAAT GG	207	P_NC002163_247_277_R	TGCGTTTAAAGCATCTCTCATAT AAT	1023
2594	ASPA_NC002163- 96692-367_402_F	TGCTGACAGACTCATGAAAAGC AA	633	GLNA_NC002163-658085- 657609_148_179_R	TGCAAAACAAAGATATATTTTGTGTC CAAA	945
2595	ASPA_NC002163- 96685-97196_1_33_F	TGCGTATGCTGCTGAGTGTATTC TACTGTTTATA	347	ASPA_NC002163-96685- 97196_467_497_R	TGAGCTTATCTACACTGGTCTGA AAG	960
2596	ASPA_NC002163- 96685-97196_1_33_F	TGCGTATGATGCTGAGTATGAC AAGAGT	502	ASPA_NC002163-96685- 97196_95_127_R	TGAGACTTGGATATATAGATGAGA TMAAT	880
2597	ASPA_NC002163- 96685-	TGAGACAGGATATATGCTATG ATTATCC	540	ASPA_NC002163-96685- 97196_188_210_R	TGAGTCCGCTATTTGAGTGGCTC TAG	872

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2598	97196_85_117_P PBM_NC002163- 327746- 328270_165 195 P	TGCGAGCTAGATATAGTACTGATTAATC GATAC	563	PBM_NC002163-327746- 328270_230 261 R	TGCGAGCTAAATTTGGATGAGCGGTAG GAAA	975
2599	PBM_NC002163- 327746- 328270_252 286 P	TGCGCTCGGTGTTTACGGAATATTC TTATATATG	593	PBM_NC002163-327746- 328270_353 381 R	TTTGTCTCGATCTCGATGAAGCTAA A	1443
2600	PBM_NC002163- 327746- 328270_1 30 P	TGCGAATGAAAGAGTTCTTTTATCC ATGA	577	PBM_NC002163-327746- 328270_95 123 R	TGATAAAGCACTAAGCGATGAAACAG C	1178
2601	PBM_NC002163- 328270_220 250 P	TBAACAGCGCTTCTCCATGCGCTATC CAAT	146	PBM_NC002163-327746- 328270_314 345 R	TCAAGTCTTTTACTTCTATGATTTAA GCTC	963
2602	UNCA_NC002163- 112166- 112647_123 152 P	TGACGATATCGGAAATGCTTTGAA TTTT	628	UNCA_NC002163-112166- 112647_199 229 R	TGCTTGTCTTTTGAGCGAGCTTGAAAG AAG	1258
2603	UNCA_NC002163- 112647_333 365 P	TGCGAGTGGACAAATTTCTTGAGAAA GCAATTT	313	UNCA_NC002163-112166- 112647_430 461 R	TCCGAAGCTGTTTGTAGCTTTAATTT GAGC	1031
2734	GYBA_AY291534_237_2 64_P	TGACGCTCATGTAATTCAGCTGTTT AT	265	GYBA_AY291534_268 288 R	TTGCGCTACTGACTACATCGT TGCAATGATGACTGCTGTTTCTTAACA GC	1407
2735	GYBA_AY291534_234_2 52_P	TATTCGGTAATGATGACCTCATGTT GAT	167	GYBA_AY291534_256 285 R	TTGCGCTACTGACTACATCGT TTGCGCTACTGACTACATCGT	1407
2736	GYBA_AY291534_170_1 98_P	TAGGAATACGCTGATTAAGCGGTAT AAA	221	GYBA_AY291534_268 288 R	TTGCGCTACTGACTACATCGT TTGCGCTACTGACTACATCGT	935
2737	GYBA_AY291534_234_2 52_P	TATTCGGTAATGATGACCTCATGTT GAT	167	GYBA_AY291534_319 346 R	TTTTCGAGCATCCAAAGTTACATCGCC GAT	1142
2738	GYBA_NC002953-7005- 8668_221 249 P	TGAGGTATGACGGAATTAATCAATA GAT	163	GYBA_NC002953-7005- 8668_265 287 R	TCTTGAGCATGACTACATCGTGC GAT	912
2739	GYBA_NC002953-7005- 8668_221 249 P	TGAGGTATGACGGAATTAATCAATA GAT	171	GYBA_NC002953-7005- 8668_316 343 R	TATCCATTAACCAAAAGTTACTTGGCC GAT	912
2740	GYBA_NC002953-7005- 8668_221 249 P	TGAGGTATGACGGAATTAATCAATA GAT	171	GYBA_NC002953-7005- 8668_253 283 R	TAGCCATGACGATGACTGCTCTTAATA AGA	1142
2741	GYBA_NC002953-7005- 8668_234 261 P	TGACCTCATGTTGATGACTCATTTT AT	264	GYBA_NC002953-7005- 8668_265 287 R	TTGTTGAGCATGACTACATCGC GAT	1299
2842	CAPC_AF188935- 56074- 56074_271 304 P	TGGAATATATTTATCTGTTGATATCC ATTGAGA	578	CAPC_AF188935-56074- 55628_346 378 R	TGTTTACGCTGCTCTTGATATGATTT GCA	1314
2843	CAPC_AF188935- 56074- 56074_271 302 P	TGATATATATATCTGTTGATGCTGCT ATTTGAG	476	CAPC_AF188935-56074- 55628_349 377 R	TTTTCGAGCTGCTCTTGATGATGATTT GATTTTC	1344
2844	CAPC_AF188935- 56074- 56074_268 303 P	TGAGGTATGATGATGATGATGATGAT GCAATGAG	331	CAPC_AF188935-56074- 55628_349 384 R	TGTTTATGATGATGATGATGATGATTT GATTTTC	860
2845	CAPC_AF188935- 56074- 56074_268 303 P	TGAGGTATGATGATGATGATGATGAT GCAATGAG	331	CAPC_AF188935-56074- 55628_349 384 R	TGTTTATGATGATGATGATGATGATTT GATTTTC	860

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2962	PPS_NC002516-1915014-1915383 240 258 F	TGCGCATCTGTCACACCG	365	PPS_NC002516-1915014-1915383 341 360 R	TGCTGCGCATCTGCGAGAT	1052
2963	TRP_NC002516-671831-672273 24 42 F	TGCTGCTGTAACGGTGCGGGA	527	TRP_NC002516-671831-672273 131 150 R	TGCGATCTCTTGCGGTGCGA	1071
2964	TRP_NC002516-671831-672273 261 292 F	TGCGCATCTGCTGCGACGTC	490	TRP_NC002516-671831-672273 282 303 R	TGATCTGCTGCGCGCGGATCT	1182
2972	OTF007_1007_1034 F	TGCGCATCTGCTGCGACGTC	592	OTF007_1126 1153 R	TGATCTGCTGCGACGTCGCGGATCT	924
2993	OMPU_NC002505-674528-675880 428 455 F	TTCGCGCATCTGCTGCGTACAC	667	OMPU_NC002505 544 567 R	TGCGTCGCGCAAAACGCTAGCTTC	1094
2994	GAFA_NC002505-506780-507937 691 721 F	TGCTCATGTAACGTAACAGTGAT	335	GAFA_NC002505-506780-507937 769 802 R	TTTTGCTTTTATGCGACTTGTATGATCAC	1442
2995	GAFA_NC002505-506780-507937 691 721 2 F	TGCTCATGTAACGTAACAGTGAT	339	GAFA_NC002505-506780-507937 769 803 R	TGCTGCTTTTATGCGACTTGTATGCGA	1008
2996	GAFA_NC002505-506780-507937 682 721 F	TGCTCATGTAACGTAACAGTGAT	396	GAFA_NC002505-506780-507937 785 817 R	TGCGAAATATCTTTCGATACCTTTATG	1085
2997	GAFA_NC002505-506780-507937 691 721 3 F	TGCTCATGTAACGTAACAGTGAT	337	GAFA_NC002505-506780-507937 785 817 R	TGCGAAATATCTTTCGATACCTTTATG	1085
2998	GAFA_NC002505-506780-507937 691 721 4 F	TGCTCATGTAACGTAACAGTGAT	336	GAFA_NC002505-506780-507937 784 817 R	TGCGAAATATCTTTCGATACCTTTATG	1087
2999	GAFA_NC002505-506780-507937 691 721 5 F	TGCTCATGTAACGTAACAGTGAT	340	GAFA_NC002505-506780-507937 784 817 2 R	TGCGAAATATCTTTCGATACCTTTATG	1086
3000	GAFA_NC002505-506780-507937 691 721 6 F	TGCTCATGTAACGTAACAGTGAT	338	GAFA_NC002505-506780-507937 769 805 R	TTCGATACCTTTTATGCGACTTTCGATC	1430
3001	CTMX_NC002505-1565967-1567341 46 71 F	TGCGCATGTAACGTAACAGTGAT	275	CTMX_NC002505-1565967-1567341 139 163 R	TTCGCGGTAGGAGTTCGTATGCGA	1025
3002	CTMX_NC002505-1565967-1567341 46 70 F	TGCGCATGTAACGTAACAGTGAT	274	CTMX_NC002505-1565967-1567341 132 162 R	TTCGCGGTAGGAGTTCGTATGCGAAT	1018
3003	CTMX_NC002505-1565967-1567341 46 70 F	TGCGCATGTAACGTAACAGTGAT	274	CTMX_NC002505-1565967-1567341 118 350 R	TGCGGTAGGAGTTCGTATGCGAAT	1225
3004	CTMX_NC002505-1565967-1567341 46 70 F	TGCGCATGTAACGTAACAGTGAT	180	CTMX_NC002505-1565967-1567341 118 350 R	TGCGGTAGGAGTTCGTATGCGAAT	982

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616222_694_F	TUPE_NCO02758- 616222_694_F	TGCGTGTGAGAGTGTGCAAT	503	TUPE_NCO02758-615038- 616222_783_813_R	TGCTTCAGCGTAGCTAATTAATTAAGG AAC	1255
3005	TUPE_NCO02758- 615038- 616222_700_726_F	TGCTGTCAATCAATGTTGAGAGAA A	638	TUPE_NCO02758-615038- 616222_778_807_R	TGCTAGCTCTAATTAATTAATTAAGCAATT TC	1238
3006	TUPE_NCO02758- 615038- 616222_702_726_F	TGCTCAATCAATGTTGTGTGAGAA	607	TUPE_NCO02758-615038- 616222_778_807_R	TGCTAGCTCTAATTAATTAATTAAGCAACT TC	1238
3007	TUPE_NCO02758- 615038- 616222_696_726_F	TGAGCTGTGTCAATCAATGAGTGTGTC AAGAA	431	TUPE_NCO02758-615038- 616222_785_818_R	TCACACCTTCAGCGTAATCTAATTAATT TAGGAA	970
3008	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3009	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3010	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3011	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3012	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3013	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3014	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3015	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3016	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3017	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3018	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3019	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3020	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3021	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134
3022	TUPE_NCO02758- 615038- 616222_700_716_P	TGCTGTGTGAAGTGTGTGTCAATCAAGAA T	386	TUPE_NCO02758-615038- 616222_778_812_R	TGCTACACCTTAATTAATTAATTAATTAAGAA AACTTC	1134

[370] Primer pair name codes and reference sequences are shown in Table 3. The primer name code typically represents the gene to which the given primer pair is targeted. The primer pair name may include specific coordinates with respect to a reference sequence defined by an extraction of a section of sequence or defined by a GenBank gi number, or the corresponding complementary sequence of the extraction, or the entire GenBank gi number as indicated by the label "no extraction." Where "no extraction" is indicated for a reference sequence, the coordinates of a primer pair named to the reference sequence are with respect to the GenBank gi listing. Gene abbreviations are shown in bold type in the "Gene Name" column.

[371] To determine the exact primer hybridization coordinates of a given pair of primers on a given bioagent nucleic acid sequence and to determine the sequences, molecular masses and base compositions of an amplification product to be obtained upon amplification of nucleic acid of a known bioagent with known sequence information in the region of interest with a given pair of primers, one with ordinary skill in bioinformatics is capable of obtaining alignments of the primers of the present invention with the GenBank gi number of the relevant nucleic acid sequence of the known bioagent. For example, the reference sequence GenBank gi numbers (Table 3) provide the identities of the sequences which can be obtained from GenBank. Alignments can be done using a bioinformatics tool such as BLASTn provided to the public by NCBI (Bethesda, MD). Alternatively, a relevant GenBank sequence may be downloaded and imported into custom programmed or commercially available bioinformatics programs wherein the alignment can be carried out to determine the primer hybridization coordinates and the sequences, molecular masses and base compositions of the amplification product. For example, to obtain the hybridization coordinates of primer pair number 2095 (SEQ ID NOs: 456:1261), First the forward primer (SEQ ID NO: 456) is subjected to a BLASTn search on the publicly available NCBI BLAST website. "RefSeq_Genomic" is chosen as the BLAST database since the gi numbers refer to genomic sequences. The BLAST query is then performed. Among the top results returned is a match to GenBank gi number 21281729 (Accession Number NC_003923). The result shown below, indicates that the forward primer hybridizes to positions 1530282..1530307 of the genomic sequence of *Staphylococcus aureus* subsp. *aureus* MW2 (represented by gi number 21281729).

Staphylococcus aureus subsp. *aureus* MW2, complete genome
Length=2820462

Features in this part of subject sequence:

Panton-Valentine leukocidin chain F precursor

Score = 52.0 bits (26), Expect = 2e-05

Identities = 26/26 (100%), Gaps = 0/26 (0%)

Strand=Plus/Plus

```

Query   1          TGAGCTGCATCAACTGTATTGGATAG   26
          |||||
Sbjct   1530282    TGAGCTGCATCAACTGTATTGGATAG   1530307

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[372] The hybridization coordinates of the reverse primer (SEQ ID NO: 1261) can be determined in a similar manner and thus, the bioagent identifying amplicon can be defined in terms of genomic coordinates. The query/subject arrangement of the result would be presented in Strand = Plus/Minus format because the reverse strand hybridizes to the reverse complement of the genomic sequence. The preceding sequence analyses are well known to one with ordinary skill in bioinformatics and thus, Table 3 contains sufficient information to determine the primer hybridization coordinates of any of the primers of Table 2 to the applicable reference sequences described therein.

Table 3: Primer Name Codes and Reference Sequence

Primer name code	Gene Name	Organism	Reference GenBank gi number
16S_EC	16S rRNA (16S ribosomal RNA gene)	<i>Escherichia coli</i>	16127994
23S_EC	23S rRNA (23S ribosomal RNA gene)	<i>Escherichia coli</i>	16127994
CAPC_BA	capC (capsule biosynthesis gene)	<i>Bacillus anthracis</i>	6470151
CYA_BA	cya (cyclic AMP gene)	<i>Bacillus anthracis</i>	4894216
DNAK_EC	dnaK (chaperone dnaK gene)	<i>Escherichia coli</i>	16127994
GROL_EC	groL (chaperonin groL)	<i>Escherichia coli</i>	16127994
HFLB_EC	hflB (cell division protein peptidase ftsH)	<i>Escherichia coli</i>	16127994
INF3_EC	inf3 (protein chain initiation factor inf3 gene)	<i>Escherichia coli</i>	16127994
LEF_BA	lef (lethal factor)	<i>Bacillus anthracis</i>	21392688
PAG_BA	pag (protective antigen)	<i>Bacillus anthracis</i>	21392688
RPLB_EC	rplB (50S ribosomal protein L2)	<i>Escherichia coli</i>	16127994
RPOB_EC	rpoB (DNA-directed RNA polymerase beta chain)	<i>Escherichia coli</i>	6127994
RPOC_EC	rpoC (DNA-directed RNA polymerase beta' chain)	<i>Escherichia coli</i>	16127994
SP101FT_SPET_11	Artificial Sequence Concatenation comprising: gki (glucose kinase) gtr (glutamine transporter protein) murI (glutamate racemase) mutS (DNA mismatch repair protein) xpt (xanthine phosphoribosyl transferase) yqiL (acetyl-CoA-acetyl transferase) tkt (transketolase)	Artificial Sequence* - partial gene sequences of <i>Streptococcus pyogenes</i>	15674250
SSPE_BA	sspE (small acid-soluble spore protein)	<i>Bacillus anthracis</i>	30253828
TUFD_EC	tufB (Elongation factor Tu)	<i>Escherichia coli</i>	16127994

VALS EC	valS (Valyl-tRNA synthetase)	<i>Escherichia coli</i>	16127994
ASPS EC	aspS (Aspartyl-tRNA synthetase)	<i>Escherichia coli</i>	16127994
CAF1 AF053947	cafI (capsular protein cafI)	<i>Yersinia pestis</i>	2996286
INV U22457	inv (invasin)	<i>Yersinia pestis</i>	1256565
LL NC003143	<i>Y. pestis</i> specific chromosomal genes - difference region	<i>Yersinia pestis</i>	16120353
BONTA X52066	BoNT/A (neurotoxin type A)	<i>Clostridium botulinum</i>	40381
MECA Y14051	mecA methicillin resistance gene	<i>Staphylococcus aureus</i>	2791983
TRPE AY094355	trpE (anthranilate synthase (large component))	<i>Acinetobacter baumannii</i>	20853695
RECA AF251469	recA (recombinase A)	<i>Acinetobacter baumannii</i>	9965210
GYRA AF100557	gyrA (DNA gyrase subunit A)	<i>Acinetobacter baumannii</i>	4240540
GYRB AB008700	gyrB (DNA gyrase subunit B)	<i>Acinetobacter baumannii</i>	4514436
WAAA Z96925	waaA (3-deoxy-D-manno-octulosonic-acid transferase)	<i>Acinetobacter baumannii</i>	2765828
CJST_CJ	Artificial Sequence Concatenation comprising: tkk (transketolase) glyA (serine hydroxymethyltransferase) gltA (citrate synthase) aspA (aspartate ammonia lyase) glnA (glutamine synthase) pgm (phosphoglycerate mutase) uncA (ATP synthetase alpha chain)	Artificial Sequence* - partial gene sequences of <i>Campylobacter jejuni</i>	15791399
RNASEP BDP	RNase P (ribonuclease P)	<i>Bordetella pertussis</i>	33591275
RNASEP BKM	RNase P (ribonuclease P)	<i>Burkholderia mallei</i>	53723370
RNASEP BS	RNase P (ribonuclease P)	<i>Bacillus subtilis</i>	16077068
RNASEP CLB	RNase P (ribonuclease P)	<i>Clostridium perfringens</i>	18308982
RNASEP EC	RNase P (ribonuclease P)	<i>Escherichia coli</i>	16127994
RNASEP RKP	RNase P (ribonuclease P)	<i>Rickettsia prowazekii</i>	15603881
RNASEP SA	RNase P (ribonuclease P)	<i>Staphylococcus aureus</i>	15922990
RNASEP VBC	RNase P (ribonuclease P)	<i>Vibrio cholerae</i>	15640032
ICD CXB	icd (isocitrate dehydrogenase)	<i>Coxiella burnetii</i>	29732244
IS1111A	multi-locus IS1111A insertion element	<i>Acinetobacter baumannii</i>	29732244
OMPA AY485227	ompA (outer membrane protein A)	<i>Rickettsia prowazekii</i>	40287451
OMP B RKP	ompB (outer membrane protein B)	<i>Rickettsia prowazekii</i>	15603881
GLTA RKP	gltA (citrate synthase)	<i>Vibrio cholerae</i>	15603881
TOXR VBC	toxR (transcription regulator toxR)	<i>Francisella tularensis</i>	15640032
ASD FRT	asd (Aspartate semialdehyde dehydrogenase)	<i>Francisella tularensis</i>	56707187
GALE FRT	galE (UDP-glucose 4-epimerase)	<i>Shigella flexneri</i>	56707187
IPAH SGF	ipah (invasion plasmid antigen)	<i>Campylobacter jejuni</i>	30061571
HUPB CJ	hupB (DNA-binding protein Hu-beta)	<i>Coxiella burnetii</i>	15791399
AB MLST	Artificial Sequence Concatenation comprising: trpE (anthranilate synthase component)	Artificial Sequence* - partial gene	Sequenced in-house (SEQ ID NO: 1444)

	I)) adk (adenylate kinase) mutY (adenine glycosylase) fumC (fumarate hydratase) efp (elongation factor p) ppa (pyrophosphate phospho- hydratase)	sequences of <i>Acinetobacter baumannii</i>	
MUPR X75439	mupB (mupirocin resistance gene)	<i>Staphylococcus aureus</i>	438226
PARC X95819	parC (topoisomerase IV)	<i>Acinetobacter baumannii</i>	1212748
SED M28521	sed (enterotoxin D)	<i>Staphylococcus aureus</i>	1492109
PLA AF053945	pla (plasminogen activator)	<i>Yersinia pestis</i>	2996216
SEJ AF053140	sej (enterotoxin J)	<i>Staphylococcus aureus</i>	3372540
GYRA NC000912	gyrA (DNA gyrase subunit A)	<i>Mycoplasma pneumoniae</i>	13507739
ACS NC002516	acsA (Acetyl CoA Synthase)	<i>Pseudomonas aeruginosa</i>	15595198
ARO NC002516	aroE (shikimate 5-dehydrogenase)	<i>Pseudomonas aeruginosa</i>	15595198
GUA NC002516	guaA (GMP synthase)	<i>Pseudomonas aeruginosa</i>	15595198
MUT NC002516	mutL (DNA mismatch repair protein)	<i>Pseudomonas aeruginosa</i>	15595198
NUO NC002516	nuoD (NADH dehydrogenase I chain C, D)	<i>Pseudomonas aeruginosa</i>	15595198
PPS NC002516	ppsA (Phosphoenolpyruvate synthase)	<i>Pseudomonas aeruginosa</i>	15595198
TRP NC002516	trpE (Anthranilate synthetase component I)	<i>Pseudomonas aeruginosa</i>	15595198
OMP2 NC000117	ompB (outer membrane protein B)	<i>Chlamydia trachomatis</i>	15604717
OMPA NC000117	ompA (outer membrane protein B)	<i>Chlamydia trachomatis</i>	15604717
GYRA NC000117	gyrA (DNA gyrase subunit A)	<i>Chlamydia trachomatis</i>	15604717
CTXA NC002505	ctxA (Cholera toxin A subunit)	<i>Vibrio cholerae</i>	15640032
CTXB NC002505	ctxB (Cholera toxin B subunit)	<i>Vibrio cholerae</i>	15640032
FUR NC002505	fur (ferric uptake regulator protein)	<i>Vibrio cholerae</i>	15640032
GAPA NC 002505	gapA (glyceraldehyde-3-phosphate dehydrogenase)	<i>Vibrio cholerae</i>	15640032
GYRB NC002505	gyrB (DNA gyrase subunit B)	<i>Vibrio cholerae</i>	15640032
OMPU NC002505	ompU (outer membrane protein)	<i>Vibrio cholerae</i>	15640032
TCPA NC002505	tcpA (toxin-coregulated pilus)	<i>Vibrio cholerae</i>	15640032
ASPA NC002163	aspA (aspartate ammonia lyase)	<i>Campylobacter jejuni</i>	15791399
GLNA NC002163	glnA (glutamine synthetase)	<i>Campylobacter jejuni</i>	15791399
GLTA NC002163	gltA (glutamate synthase)	<i>Campylobacter jejuni</i>	15791399
GLYA NC002163	glyA (serine hydroxymethyltransferase)	<i>Campylobacter jejuni</i>	15791399
PGM NC002163	pgm (phosphoglyceromutase)	<i>Campylobacter jejuni</i>	15791399
TKT NC002163	tkt (transketolase)	<i>Campylobacter jejuni</i>	15791399
UNCA NC002163	uncA (ATP synthetase alpha chain)	<i>Campylobacter jejuni</i>	15791399
AGR-III NC003923	agr-III (accessory gene regulator-III)	<i>Staphylococcus aureus</i>	21281729
AROC NC003923	aroC (carbamate kinase)	<i>Staphylococcus aureus</i>	21281729
AROE NC003923	aroE (shikimate 5-dehydrogenase)	<i>Staphylococcus</i>	21281729

		aureus	
BSA-A NC003923	bsa-a (glutathione peroxidase)	Staphylococcus aureus	21281729
BSA-B NC003923	bsa-b (epidermin biosynthesis protein EpiB)	Staphylococcus aureus	21281729
GLPF NC003923	glpF (glycerol transporter)	Staphylococcus aureus	21281729
GMK NC003923	gmK (guanylate kinase)	Staphylococcus aureus	21281729
MECI-R NC003923	mecR1 (truncated methicillin resistance protein)	Staphylococcus aureus	21281729
PTA NC003923	pta (phosphate acetyltransferase)	Staphylococcus aureus	21281729
PVLUK NC003923	pvluk (Panton-Valentine leukocidin chain F precursor)	Staphylococcus aureus	21281729
SA442 NC003923	sa442 gene	Staphylococcus aureus	21281729
SEA NC003923	sea (staphylococcal enterotoxin A precursor)	Staphylococcus aureus	21281729
SEC NC003923	sec4 (enterotoxin type C precursor)	Staphylococcus aureus	21281729
TPI NC003923	tpi (triosephosphate isomerase)	Staphylococcus aureus	21281729
YQI NC003923	yqi (acetyl-CoA C-acetyltransferase homologue)	Staphylococcus aureus	21281729
GALE AF513299	galE (galactose epimerase)	Francisella tularensis	23506418
VVHA NC004460	vvha (cytotoxin, cytotoxin precursor)	Vibrio vulnificus	27366463
TDH NC004605	tdh (thermostable direct hemolysin A)	Vibrio parahaemolyticus	28899855
AGR-II NC002745	agr-II (accessory gene regulator-II)	Staphylococcus aureus	29165615
PARC NC003997	parC (topoisomerase IV)	Bacillus anthracis	30260195
GYRA AY291534	gyrA (DNA gyrase subunit A)	Bacillus anthracis	31323274
AGR-I AJ617706	agr-I (accessory gene regulator-I)	Staphylococcus aureus	46019543
AGR-IV AJ617711	agr-IV (accessory gene regulator-III)	Staphylococcus aureus	46019563
BLA3 NC002952	bla3 (beta lactamase III)	Staphylococcus aureus	49482253
ERMA NC002952	erma (rRNA methyltransferase A)	Staphylococcus aureus	49482253
ERMB Y13600	ermB (rRNA methyltransferase B)	Staphylococcus aureus	49482253
SEA-SEE NC002952	sea (staphylococcal enterotoxin A precursor)	Staphylococcus aureus	49482253
SEA-SEE NC002952	sea (staphylococcal enterotoxin A precursor)	Staphylococcus aureus	49482253
SEE NC002952	sea (staphylococcal enterotoxin A precursor)	Staphylococcus aureus	49482253
SEH NC002953	seh (staphylococcal enterotoxin H)	Staphylococcus aureus	49484912
ERMC NC005908	ermC (rRNA methyltransferase C)	Staphylococcus aureus	49489772
MUTS AY698802	mutS (DNA mismatch repair protein)	Shigella boydii	52698233
NUC NC002758	nuc (staphylococcal nuclease)	Staphylococcus aureus	57634611
SEB NC002758	seb (enterotoxin type B precursor)	Staphylococcus aureus	57634611
SEG NC002758	seg (staphylococcal enterotoxin G)	Staphylococcus aureus	57634611
SEI NC002758	sei (staphylococcal enterotoxin I)	Staphylococcus aureus	57634611
TSST NC002758	tsst (toxic shock syndrome toxin-1)	Staphylococcus aureus	57634611
TUFB NC002758	tufB (Elongation factor Tu)	Staphylococcus aureus	57634611

[373] Note: artificial reference sequences represent concatenations of partial gene extractions from the indicated reference gi number. Partial sequences were used to create the concatenated sequence because complete gene sequences were not necessary for primer design.

Example 2: Sample Preparation and PCR

[374] Genomic DNA was prepared from samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocols.

[375] All PCR reactions were assembled in 50 μ L reaction volumes in a 96-well microtiter plate format using a Packard MP11 liquid handling robotic platform and M.J. Dyad thermocyclers (MJ research, Waltham, MA) or Eppendorf Mastercycler thermocyclers (Eppendorf, Westbury, NY). The PCR reaction mixture consisted of 4 units of Amplitaq Gold, 1x buffer II (Applied Biosystems, Foster City, CA), 1.5 mM MgCl₂, 0.4 M betaine, 800 μ M dNTP mixture and 250 nM of each primer. The following typical PCR conditions were used: 95°C for 10 min followed by 8 cycles of 95°C for 30 seconds, 48°C for 30 seconds, and 72°C 30 seconds with the 48°C annealing temperature increasing 0.9°C with each of the eight cycles. The PCR was then continued for 37 additional cycles of 95°C for 15 seconds, 56°C for 20 seconds, and 72°C 20 seconds.

Example 3: Purification of PCR Products for Mass Spectrometry with Ion Exchange Resin-Magnetic Beads

[376] For solution capture of nucleic acids with ion exchange resin linked to magnetic beads, 25 μ L of a 2.5 mg/mL suspension of BioClone amine terminated superparamagnetic beads were added to 25 μ L of a PCR (or RT-PCR) reaction containing approximately 10 pM of a typical PCR amplification product. The above suspension was mixed for approximately 5 minutes by vortexing or pipetting, after which the liquid was removed after using a magnetic separator. The beads containing bound PCR amplification product were then washed three times with 50mM ammonium bicarbonate/50% MeOH or 100mM ammonium bicarbonate/50% MeOH, followed by three more washes with 50% MeOH. The bound PCR amplicon was eluted with a solution of 25mM piperidine, 25mM imidazole, 35% MeOH which included peptide calibration standards.

Example 4: Mass Spectrometry and Base Composition Analysis

[377] The ESI-FTICR mass spectrometer is based on a Bruker Daltonics (Billerica, MA) Apex II 70e electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer that employs an actively shielded 7 Tesla superconducting magnet. The active shielding constrains the majority of the fringing magnetic field from the superconducting magnet to a relatively small volume. Thus,

components that might be adversely affected by stray magnetic fields, such as CRT monitors, robotic components, and other electronics, can operate in close proximity to the FTICR spectrometer. All aspects of pulse sequence control and data acquisition were performed on a 600 MHz Pentium II data station running Bruker's Xmass software under Windows NT 4.0 operating system. Sample aliquots, typically 15 μ l, were extracted directly from 96-well microtiter plates using a CTC HTS PAL autosampler (LEAP Technologies, Carboro, NC) triggered by the FTICR data station. Samples were injected directly into a 10 μ l sample loop integrated with a fluidics handling system that supplies the 100 μ l/hr flow rate to the ESI source. Ions were formed via electrospray ionization in a modified Analytica (Branford, CT) source employing an off axis, grounded electrospray probe positioned approximately 1.5 cm from the metalized terminus of a glass desolvation capillary. The atmospheric pressure end of the glass capillary was biased at 6000 V relative to the ESI needle during data acquisition. A counter-current flow of dry N₂ was employed to assist in the desolvation process. Ions were accumulated in an external ion reservoir comprised of an rf-only hexapole, a skimmer cone, and an auxiliary gate electrode, prior to injection into the trapped ion cell where they were mass analyzed. Ionization duty cycles greater than 99% were achieved by simultaneously accumulating ions in the external ion reservoir during ion detection. Each detection event consisted of 1M data points digitized over 2.3 s. To improve the signal-to-noise ratio (S/N), 32 scans were co-added for a total data acquisition time of 74 s.

[378] The ESI-TOF mass spectrometer is based on a Bruker Daltonics MicroTOF™. Ions from the ESI source undergo orthogonal ion extraction and are focused in a reflectron prior to detection. The TOF and FTICR are equipped with the same automated sample handling and fluidics described above. Ions are formed in the standard MicroTOF™ ESI source that is equipped with the same off-axis sprayer and glass capillary as the FTICR ESI source. Consequently, source conditions were the same as those described above. External ion accumulation was also employed to improve ionization duty cycle during data acquisition. Each detection event on the TOF was comprised of 75,000 data points digitized over 75 μ s.

[379] The sample delivery scheme allows sample aliquots to be rapidly injected into the electrospray source at high flow rate and subsequently be electrosprayed at a much lower flow rate for improved ESI sensitivity. Prior to injecting a sample, a bolus of buffer was injected at a high flow rate to rinse the transfer line and spray needle to avoid sample contamination/carryover. Following the rinse step, the autosampler injected the next sample and the flow rate was switched to low flow. Following a brief equilibration delay, data acquisition commenced. As spectra were co-added, the autosampler continued rinsing the syringe and picking up buffer to rinse the injector and sample transfer line. In general, two syringe rinses and one injector rinse were required to minimize sample carryover. During a

routine screening protocol a new sample mixture was injected every 106 seconds. More recently a fast wash station for the syringe needle has been implemented which, when combined with shorter acquisition times, facilitates the acquisition of mass spectra at a rate of just under one spectrum/minute.

[380] Raw mass spectra were post-calibrated with an internal mass standard and deconvoluted to monoisotopic molecular masses. Unambiguous base compositions were derived from the exact mass measurements of the complementary single-stranded oligonucleotides. Quantitative results are obtained by comparing the peak heights with an internal PCR calibration standard present in every PCR well at 500 molecules per well. Calibration methods are commonly owned and disclosed in U.S. Provisional Patent Application Serial No. 60/545,425 which is incorporated herein by reference in entirety.

Example 5: *De Novo* Determination of Base Composition of Amplification Products using Molecular Mass Modified Deoxynucleotide Triphosphates

[381] Because the molecular masses of the four natural nucleobases have a relatively narrow molecular mass range (A = 313.058, G = 329.052, C = 289.046, T = 304.046 – See Table 4), a persistent source of ambiguity in assignment of base composition can occur as follows: two nucleic acid strands having different base composition may have a difference of about 1 Da when the base composition difference between the two strands is $G \leftrightarrow A$ (-15.994) combined with $C \leftrightarrow T$ (+15.000). For example, one 99-mer nucleic acid strand having a base composition of $A_{27}G_{30}C_{21}T_{21}$ has a theoretical molecular mass of 30779.058 while another 99-mer nucleic acid strand having a base composition of $A_{26}G_{31}C_{22}T_{20}$ has a theoretical molecular mass of 30780.052. A 1 Da difference in molecular mass may be within the experimental error of a molecular mass measurement and thus, the relatively narrow molecular mass range of the four natural nucleobases imposes an uncertainty factor.

[382] The present invention provides for a means for removing this theoretical 1 Da uncertainty factor through amplification of a nucleic acid with one mass-tagged nucleobase and three natural nucleobases. The term “nucleobase” as used herein is synonymous with other terms in use in the art including “nucleotide,” “deoxynucleotide,” “nucleotide residue,” “deoxynucleotide residue,” “nucleotide triphosphate (NTP),” or deoxynucleotide triphosphate (dNTP).

[383] Addition of significant mass to one of the 4 nucleobases (dNTPs) in an amplification reaction, or in the primers themselves, will result in a significant difference in mass of the resulting amplification product (significantly greater than 1 Da) arising from ambiguities arising from the $G \leftrightarrow A$ combined with $C \leftrightarrow T$ event (Table 4). Thus, the same the $G \leftrightarrow A$ (-15.994) event combined with 5-Iodo-C \leftrightarrow T (-110.900) event would result in a molecular mass difference of 126.894. If the molecular mass of the

base composition $A_{27}G_{30}5\text{-Iodo-C}_{21}T_{21}$ (33422.958) is compared with $A_{26}G_{31}5\text{-Iodo-C}_{22}T_{20}$, (33549.852) the theoretical molecular mass difference is +126.894. The experimental error of a molecular mass measurement is not significant with regard to this molecular mass difference. Furthermore, the only base composition consistent with a measured molecular mass of the 99-mer nucleic acid is $A_{27}G_{30}5\text{-Iodo-C}_{21}T_{21}$. In contrast, the analogous amplification without the mass tag has 18 possible base compositions.

Table 4: Molecular Masses of Natural Nucleobases and the Mass-Modified Nucleobase 5-Iodo-C and Molecular Mass Differences Resulting from Transitions

Nucleobase	Molecular Mass	Transition	Δ Molecular Mass
A	313.058	A \rightarrow T	-9.012
A	313.058	A \rightarrow C	-24.012
A	313.058	A \rightarrow 5-Iodo-C	101.888
A	313.058	A \rightarrow G	15.994
T	304.046	T \rightarrow A	9.012
T	304.046	T \rightarrow C	-15.000
T	304.046	T \rightarrow 5-Iodo-C	110.900
T	304.046	T \rightarrow G	25.006
C	289.046	C \rightarrow A	24.012
C	289.046	C \rightarrow T	15.000
C	289.046	C \rightarrow G	40.006
5-Iodo-C	414.946	5-Iodo-C \rightarrow A	-101.888
5-Iodo-C	414.946	5-Iodo-C \rightarrow T	-110.900
5-Iodo-C	414.946	5-Iodo-C \rightarrow G	-85.894
G	329.052	G \rightarrow A	-15.994
G	329.052	G \rightarrow T	-25.006
G	329.052	G \rightarrow C	-40.006
G	329.052	G \rightarrow 5-Iodo-C	85.894

[384] Mass spectra of bioagent-identifying amplicons were analyzed independently using a maximum-likelihood processor, such as is widely used in radar signal processing. This processor, referred to as GenX, first makes maximum likelihood estimates of the input to the mass spectrometer for each primer by running matched filters for each base composition aggregate on the input data. This includes the GenX response to a calibrant for each primer.

[385] The algorithm emphasizes performance predictions culminating in probability-of-detection versus probability-of-false-alarm plots for conditions involving complex backgrounds of naturally occurring organisms and environmental contaminants. Matched filters consist of *a priori* expectations of signal values given the set of primers used for each of the bioagents. A genomic sequence database is

used to define the mass base count matched filters. The database contains the sequences of known bacterial bioagents and includes threat organisms as well as benign background organisms. The latter is used to estimate and subtract the spectral signature produced by the background organisms. A maximum likelihood detection of known background organisms is implemented using matched filters and a running-sum estimate of the noise covariance. Background signal strengths are estimated and used along with the matched filters to form signatures which are then subtracted. The maximum likelihood process is applied to this "cleaned up" data in a similar manner employing matched filters for the organisms and a running-sum estimate of the noise-covariance for the cleaned up data.

[386] The amplitudes of all base compositions of bioagent-identifying amplicons for each primer are calibrated and a final maximum likelihood amplitude estimate per organism is made based upon the multiple single primer estimates. Models of all system noise are factored into this two-stage maximum likelihood calculation. The processor reports the number of molecules of each base composition contained in the spectra. The quantity of amplification product corresponding to the appropriate primer set is reported as well as the quantities of primers remaining upon completion of the amplification reaction.

[387] Base count blurring can be carried out as follows. "Electronic PCR" can be conducted on nucleotide sequences of the desired bioagents to obtain the different expected base counts that could be obtained for each primer pair. See for example, ncbi.nlm.nih.gov/sutils/e-pcr/; Schuler, *Genome Res.* 7:541-50, 1997. In one illustrative embodiment, one or more spreadsheets, such as Microsoft Excel workbooks contain a plurality of worksheets. First in this example, there is a worksheet with a name similar to the workbook name; this worksheet contains the raw electronic PCR data. Second, there is a worksheet named "filtered bioagents base count" that contains bioagent name and base count; there is a separate record for each strain after removing sequences that are not identified with a genus and species and removing all sequences for bioagents with less than 10 strains. Third, there is a worksheet, "Sheet1" that contains the frequency of substitutions, insertions, or deletions for this primer pair. This data is generated by first creating a pivot table from the data in the "filtered bioagents base count" worksheet and then executing an Excel VBA macro. The macro creates a table of differences in base counts for bioagents of the same species, but different strains. One of ordinary skill in the art may understand additional pathways for obtaining similar table differences without undo experimentation.

[388] Application of an exemplary script, involves the user defining a threshold that specifies the fraction of the strains that are represented by the reference set of base counts for each bioagent. The reference set of base counts for each bioagent may contain as many different base counts as are needed

to meet or exceed the threshold. The set of reference base counts is defined by taking the most abundant strain's base type composition and adding it to the reference set and then the next most abundant strain's base type composition is added until the threshold is met or exceeded. The current set of data was obtained using a threshold of 55%, which was obtained empirically.

[389] For each base count not included in the reference base count set for that bioagent, the script then proceeds to determine the manner in which the current base count differs from each of the base counts in the reference set. This difference may be represented as a combination of substitutions, $S_i=X_i$, and insertions, $I_i=Y_i$, or deletions, $D_i=Z_i$. If there is more than one reference base count, then the reported difference is chosen using rules that aim to minimize the number of changes and, in instances with the same number of changes, minimize the number of insertions or deletions. Therefore, the primary rule is to identify the difference with the minimum sum (X_i+Y_i) or (X_i+Z_i), e.g., one insertion rather than two substitutions. If there are two or more differences with the minimum sum, then the one that will be reported is the one that contains the most substitutions.

[390] Differences between a base count and a reference composition are categorized as one, two, or more substitutions, one, two, or more insertions, one, two, or more deletions, and combinations of substitutions and insertions or deletions. The different classes of nucleobase changes and their probabilities of occurrence have been delineated in U.S. Patent Application Publication No. 2004209260 (U.S. Application Serial No. 10/418,514) which is incorporated herein by reference in entirety.

Example 6: Use of Broad Range Survey and Division Wide Primer Pairs for Identification of Bacteria in an Epidemic Surveillance Investigation

[391] This investigation employed a set of 16 primer pairs which is herein designated the "surveillance primer set" and comprises broad range survey primer pairs, division wide primer pairs and a single *Bacillus* clade primer pair. The surveillance primer set is shown in Table 5 and consists of primer pairs originally listed in Table 2. This surveillance set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row. Primer pair 449 (non-T modified) has been modified twice. Its predecessors are primer pairs 70 and 357, displayed below in the same row. Primer pair 360 has also been modified twice and its predecessors are primer pairs 17 and 118.

Table 5: Bacterial Primer Pairs of the Surveillance Primer Set

Primer Pair	Forward Primer Name	Forward Primer	Reverse Primer Name	Reverse Primer	Target Gene
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No.		(SEQ ID NO:)		(SEQ ID NO:)	
346	16S_EC_713_732_TM0D_F	202	16S_EC_789_809_TM0D_R	1110	16S rRNA
10	16S_EC_713_732_F	21	16S_EC_789_809	798	16S rRNA
347	16S_EC_785_806_TM0D_F	560	16S_EC_880_897_TM0D_R	1278	16S rRNA
11	16S_EC_785_806_F	118	16S_EC_880_897_R	830	16S rRNA
348	16S_EC_960_981_TM0D_F	706	16S_EC_1054_1073_TM0D_R	895	16S rRNA
14	16S_EC_960_981_F	672	16S_EC_1054_1073_R	735	16S rRNA
349	23S_EC_1826_1843_TM0D_F	401	23S_EC_1906_1924_TM0D_R	1156	23S rRNA
16	23S_EC_1826_1843_F	80	23S_EC_1906_1924_R	805	23S rRNA
352	INFB_EC_1365_1393_TM0D_F	687	INFB_EC_1439_1467_TM0D_R	1411	infB
34	INFB_EC_1365_1393_F	524	INFB_EC_1439_1467_R	1248	infB
354	RPOC_EC_2218_2241_TM0D_F	405	RPOC_EC_2313_2337_TM0D_R	1072	rpoC
52	RPOC_EC_2218_2241_F	81	RPOC_EC_2313_2337_R	790	rpoC
355	SSPE_BA_115_137_TM0D_F	255	SSPE_BA_197_222_TM0D_R	1402	sspE
58	SSPE_BA_115_137_F	45	SSPE_BA_197_222_R	1201	sspE
356	RPLB_EC_650_679_TM0D_F	232	RPLB_EC_739_762_TM0D_R	592	rplB
66	RPLB_EC_650_679_F	98	RPLB_EC_739_762_R	999	rplB
358	VALS_EC_1105_1124_TM0D_F	385	VALS_EC_1195_1218_TM0D_R	1093	vals
71	VALS_EC_1105_1124_F	77	VALS_EC_1195_1218_R	795	vals
359	RPOB_EC_1845_1866_TM0D_F	659	RPOB_EC_1909_1929_TM0D_R	1250	rpoB
72	RPOB_EC_1845_1866_F	233	RPOB_EC_1909_1929_R	825	rpoB
360	23S_EC_2646_2667_TM0D_F	409	23S_EC_2745_2765_TM0D_R	1434	23S rRNA
118	23S_EC_2646_2667_F	84	23S_EC_2745_2765_R	1389	23S rRNA
17	23S_EC_2645_2669_F	408	23S_EC_2744_2761_R	1252	23S rRNA
361	16S_EC_1090_1111_2_TM0D_F	697	16S_EC_1175_1196_TM0D_R	1398	16S rRNA
3	16S_EC_1090_1111_2_F	651	16S_EC_1175_1196_R	1359	16S rRNA
362	RPOB_EC_3799_3821_TM0D_F	581	RPOB_EC_3862_3888_TM0D_R	1325	rpoB
289	RPOB_EC_3799_3821_F	124	RPOB_EC_3862_3888_R	840	rpoB
363	RPOC_EC_2146_2174_TM0D_F	284	RPOC_EC_2227_2245_TM0D_R	898	rpoC
290	RPOC_EC_2146_2174_F	52	RPOC_EC_2227_2245_R	736	rpoC
367	TUFB_EC_957_979_TM0D_F	308	TUFB_EC_1034_1058_TM0D_R	1276	tufB
293	TUFB_EC_957_979_F	55	TUFB_EC_1034_1058_R	829	tufB
449	RPLB_EC_690_710_F	309	RPLB_EC_737_758_R	1336	rplB
357	RPLB_EC_688_710_TM0D_F	296	RPLB_EC_736_757_TM0D_R	1337	rplB
67	RPLB_EC_688_710_F	54	RPLB_EC_736_757_R	842	rplB

[392] The 16 primer pairs of the surveillance set are used to produce bioagent identifying amplicons whose base compositions are sufficiently different amongst all known bacteria at the species level to identify, at a reasonable confidence level, any given bacterium at the species level. As shown in Tables 6A-E, common respiratory bacterial pathogens can be distinguished by the base compositions of bioagent identifying amplicons obtained using the 16 primer pairs of the surveillance set. In some cases, triangulation identification improves the confidence level for species assignment. For example, nucleic acid from *Streptococcus pyogenes* can be amplified by nine of the sixteen surveillance primer pairs and *Streptococcus pneumoniae* can be amplified by ten of the sixteen surveillance primer pairs. The base

compositions of the bioagent identifying amplicons are identical for only one of the analogous bioagent identifying amplicons and differ in all of the remaining analogous bioagent identifying amplicons by up to four bases per bioagent identifying amplicon. The resolving power of the surveillance set was confirmed by determination of base compositions for 120 isolates of respiratory pathogens representing 70 different bacterial species and the results indicated that natural variations (usually only one or two base substitutions per bioagent identifying amplicon) amongst multiple isolates of the same species did not prevent correct identification of major pathogenic organisms at the species level.

[393] *Bacillus anthracis* is a well known biological warfare agent which has emerged in domestic terrorism in recent years. Since it was envisioned to produce bioagent identifying amplicons for identification of *Bacillus anthracis*, additional drill-down analysis primers were designed to target genes present on virulence plasmids of *Bacillus anthracis* so that additional confidence could be reached in positive identification of this pathogenic organism. Three drill-down analysis primers were designed and are listed in Tables 2 and 6. In Table 6, the drill-down set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row.

Table 6: Drill-Down Primer Pairs for Confirmation of Identification of *Bacillus anthracis*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO.)	Reverse Primer Name	Reverse Primer (SEQ ID NO.)	Target Gene
350	CAPC_BA_274_303_TMOD_F	476	CAPC_BA_349_376_TMOD_R	1314	capC
24	CAPC_BA_274_303_F	109	CAPC_BA_349_376_R	837	capC
351	CYA_BA_1353_1379_TMOD_F	355	CYA_BA_1448_1467_TMOD_R	1423	cyA
30	CYA_BA_1353_1379_F	64	CYA_BA_1448_1467_R	1342	cyA
353	LEF_BA_756_781_TMOD_F	220	LEF_BA_843_872_TMOD_R	1394	lef
37	LEF_BA_756_781_F	26	LEF_BA_843_872_R	1135	lef

[394] Phylogenetic coverage of bacterial space of the sixteen surveillance primers of Table 5 and the three *Bacillus anthracis* drill-down primers of Table 6 is shown in Figure 3 which lists common pathogenic bacteria. Figure 3 is not meant to be comprehensive in illustrating all species identified by the primers. Only pathogenic bacteria are listed as representative examples of the bacterial species that can be identified by the primers and methods of the present invention. Nucleic acid of groups of bacteria enclosed within the polygons of Figure 3 can be amplified to obtain bioagent identifying amplicons using the primer pair numbers listed in the upper right hand corner of each polygon. Primer coverage for polygons within polygons is additive. As an illustrative example, bioagent identifying amplicons can be obtained for *Chlamydia trachomatis* by amplification with, for example, primer pairs 346-349, 360 and

361, but not with any of the remaining primers of the surveillance primer set. On the other hand, bioagent identifying amplicons can be obtained from nucleic acid originating from *Bacillus anthracis* (located within 5 successive polygons) using, for example, any of the following primer pairs: 346-349, 360, 361 (base polygon), 356, 449 (second polygon), 352 (third polygon), 355 (fourth polygon), 350, 351 and 353 (fifth polygon). Multiple coverage of a given organism with multiple primers provides for increased confidence level in identification of the organism as a result of enabling broad triangulation identification.

[395] In Tables 7A-E, base compositions of respiratory pathogens for primer target regions are shown. Two entries in a cell, represent variation in ribosomal DNA operons. The most predominant base composition is shown first and the minor (frequently a single operon) is indicated by an asterisk (*). Entries with NO DATA mean that the primer would not be expected to prime this species due to mismatches between the primer and target region, as determined by theoretical PCR.

Table 7A – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 346, 347 and 348

Organism	Strain	Primer 346 [A G C T]	Primer 347 [A G C T]	Primer 348 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	[29 32 25 13] [29 31 25 13]*	[23 38 28 26] [23 37 28 26]*	[26 32 28 30] [26 31 28 30]*
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	[29 32 25 13]	[22 39 28 26]	[29 30 28 29] [30 30 27 29]*
<i>Yersinia pestis</i>	KIMS P12 (Biovar Mediaevalis)	[29 32 25 13]	[22 39 28 26]	[29 30 28 29] [29 30 28 29]
<i>Yersinia pestis</i>	91001	[29 32 25 13]	[22 39 28 26]	[30 30 27 29]*
<i>Haemophilus influenzae</i>	KW20	[28 31 23 17]	[24 37 25 27]	[29 30 28 29]
<i>Pseudomonas aeruginosa</i>	PAO1	[30 31 23 15]	[26 36 29 24] [27 36 29 23]*	[26 32 29 29] [25 32 29 29]
<i>Pseudomonas fluorescens</i>	Pf0-1	[30 31 23 15]	[26 35 29 25]	[28 31 28 29]
<i>Pseudomonas putida</i>	KF2440	[30 31 23 15]	[28 33 27 27]	[27 32 29 28]
<i>Legionella pneumophila</i>	Philadelphia-1	[30 30 24 15]	[33 33 23 27]	[29 28 28 31]
<i>Francisella tularensis</i>	schu 4	[32 29 22 16]	[28 38 26 26]	[25 32 28 31]
<i>Bordetella pertussis</i>	Tohama I	[30 29 24 16]	[23 37 30 24]	[30 32 30 26]
<i>Burkholderia cepacia</i>	J2315	[29 29 27 14]	[27 32 26 29]	[27 36 31 24] [20 42 35 19]*
<i>Burkholderia pseudomallei</i>	K96243	[29 29 27 14]	[27 32 26 29]	[27 36 31 24]
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	[29 28 24 18]	[27 34 26 28]	[24 36 29 27]
<i>Neisseria meningitidis</i>	MC58 (serogroup B)	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
<i>Neisseria meningitidis</i>	serogroup C, FAM18	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
<i>Neisseria meningitidis</i>	Z2491 (serogroup A)	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
<i>Chlamydia pneumoniae</i>	TW-183	[31 27 22 19]	NO DATA	[32 27 27 29]

<i>Chlamydia pneumoniae</i>	AR39	[31 27 22 19]	NO DATA	[32 27 27 29]
<i>Chlamydia pneumoniae</i>	CWL029	[31 27 22 19]	NO DATA	[32 27 27 29]
<i>Chlamydia pneumoniae</i>	J138	[31 27 22 19]	NO DATA	[32 27 27 29]
<i>Corynebacterium diphtheriae</i>	NCTC13129	[29 34 21 15]	[22 38 31 25]	[22 33 25 34]
<i>Mycobacterium avium</i>	k10	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycobacterium avium</i>	104	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycobacterium tuberculosis</i>	CSU#93	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycobacterium tuberculosis</i>	CDC 1551	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycoplasma pneumoniae</i>	M129	[31 29 19 20]	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [29 31 30 29] *
<i>Staphylococcus aureus</i>	MSSA476	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30] *
<i>Staphylococcus aureus</i>	COL	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30] *
<i>Staphylococcus aureus</i>	Mu50	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30] *
<i>Staphylococcus aureus</i>	MW2	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30] *
<i>Staphylococcus aureus</i>	N315	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30] *
<i>Staphylococcus aureus</i>	NCTC 8325	[27 30 21 21]	[25 35 30 26] [25 35 31 26] *	[30 29 30 29] [30 29 29 30]
<i>Streptococcus agalactiae</i>	NEM316	[26 32 23 18]	[24 36 31 25] [24 36 30 26] *	[25 32 29 30]
<i>Streptococcus equi</i>	NC 002955	[26 32 23 18]	[23 37 31 25]	[29 30 25 32]
<i>Streptococcus pyogenes</i>	MGAS8232	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	MGAS315	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	SSI-1	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	MGAS10394	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	Manfredo (M5)	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	SF370 (M1)	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pneumoniae</i>	670	[26 32 23 18]	[25 35 28 28]	[25 32 29 30]
<i>Streptococcus pneumoniae</i>	R6	[26 32 23 18]	[25 35 28 28]	[25 32 29 30]
<i>Streptococcus pneumoniae</i>	TIGR4	[26 32 23 18]	[25 35 28 28]	[25 32 30 29]
<i>Streptococcus gordonii</i>	NCTC7868	[25 33 23 18]	[24 36 31 25]	[25 31 29 31]
<i>Streptococcus mitis</i>	NCTC 12261	[26 32 23 18]	[25 35 30 26]	[25 32 29 30] [24 31 35 29] *
<i>Streptococcus mutans</i>	UA159	[24 32 24 19]	[25 37 30 24]	[28 31 26 31]

Table 7B – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying

Amplicons Corresponding to Primer Pair Nos: 349, 360, and 356

Organism	Strain	Primer 349	Primer 360	Primer 356
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		[A G C T]	[A G C T]	[A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	[25 31 25 22]	[33 37 25 27]	NO DATA
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	[25 31 27 20]		
		[25 32 26 20]*	[34 35 25 28]	NO DATA
<i>Yersinia pestis</i>	KIMS P12 (Biovar Mediaevalis)	[25 31 27 20]		
		[25 32 26 20]*	[34 35 25 28]	NO DATA
<i>Yersinia pestis</i>	91001	[25 31 27 20]	[34 35 25 28]	NO DATA
<i>Haemophilus influenzae</i>	KW20	[28 28 25 20]	[32 38 25 27]	NO DATA
<i>Pseudomonas aeruginosa</i>	PAO1	[24 31 26 20]	[31 36 27 27]	
			[31 36 27 28]*	NO DATA
<i>Pseudomonas fluorescens</i>	PF0-1	NO DATA	[30 37 27 28]	
			[30 37 27 28]	NO DATA
<i>Pseudomonas putida</i>	KT2440	[24 31 26 20]	[30 37 27 28]	NO DATA
<i>Legionella pneumophila</i>	Philadelphia-1	[23 30 25 23]	[30 39 29 24]	NO DATA
<i>Francisella tularensis</i>	schu 4	[26 31 25 19]	[32 36 27 27]	NO DATA
<i>Bordetella pertussis</i>	Tohama I	[21 29 24 18]	[33 36 26 27]	NO DATA
<i>Burkholderia cepacia</i>	J2315	[23 27 22 20]	[31 37 28 26]	NO DATA
<i>Burkholderia pseudomallei</i>	K96243	[23 27 22 20]	[31 37 28 26]	NO DATA
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	[24 27 24 17]	[34 37 25 26]	NO DATA
<i>Neisseria meningitidis</i>	MC58 (serogroup B)	[25 27 22 18]	[34 37 25 26]	NO DATA
<i>Neisseria meningitidis</i>	serogroup C, FAM18	[25 26 23 18]	[34 37 25 26]	NO DATA
<i>Neisseria meningitidis</i>	Z2491 (serogroup A)	[25 26 23 18]	[34 37 25 26]	NO DATA
<i>Chlamydia pneumoniae</i>	TW-183	[30 28 27 18]	NO DATA	NO DATA
<i>Chlamydia pneumoniae</i>	AR39	[30 28 27 18]	NO DATA	NO DATA
<i>Chlamydia pneumoniae</i>	CWL029	[30 28 27 18]	NO DATA	NO DATA
<i>Chlamydia pneumoniae</i>	J138	[30 28 27 18]	NO DATA	NO DATA
<i>Corynebacterium diphtheriae</i>	NCTC13129	NO DATA	[29 40 28 25]	NO DATA
<i>Mycobacterium avium</i>	x10	NO DATA	[33 35 32 22]	NO DATA
<i>Mycobacterium avium</i>	104	NO DATA	[33 35 32 22]	NO DATA
<i>Mycobacterium tuberculosis</i>	CSU#93	NO DATA	[30 36 34 22]	NO DATA
<i>Mycobacterium tuberculosis</i>	CDC 1551	NO DATA	[30 36 34 22]	NO DATA
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	NO DATA	[30 36 34 22]	NO DATA
<i>Mycoplasma pneumoniae</i>	MI29	[28 30 24 19]	[34 31 29 28]	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	MRSA476	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	COL	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	Mu50	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	MW2	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	N315	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus</i>	NCTC 8325	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]

<i>aureus</i>				
<i>Streptococcus agalactiae</i>	NEM316	[28 31 22 20]	[33 37 24 28]	[37 30 28 26]
<i>Streptococcus equi</i>	NC 002955	[28 31 23 19]	[33 38 24 27]	[37 31 28 25]
<i>Streptococcus pyogenes</i>	MGAS8232	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	MGAS315	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	SSI-1	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	MGAS10394	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	Manfredo (M5)	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	SF370 (M1)	[28 31 22 20] *	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pneumoniae</i>	670	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
<i>Streptococcus pneumoniae</i>	R6	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
<i>Streptococcus pneumoniae</i>	TIGR4	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
<i>Streptococcus gordonii</i>	NCTC7868	[28 32 23 20]	[34 36 24 28]	[36 31 29 25]
<i>Streptococcus mitis</i>	NCTC 12261	[28 31 22 20] [29 30 22 20] *	[34 36 24 28]	[37 30 29 25]
<i>Streptococcus mutans</i>	UA159	[26 32 23 22]	[34 37 24 27]	NO DATA

Table 7C – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 449, 354, and 352

Organism	Strain	Primer 449 [A G C T]	Primer 354 [A G C T]	Primer 352 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	NO DATA	[27 33 36 26]	NO DATA
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	NO DATA	[29 31 33 29]	[32 28 20 25]
<i>Yersinia pestis</i>	KIM5 F12 (Biovar Mediaevalis)	NO DATA	[29 31 33 29]	[32 28 20 25]
<i>Yersinia pestis</i>	91001	NO DATA	[29 31 33 29]	NO DATA
<i>Haemophilus influenzae</i>	KW20	NO DATA	[30 29 31 32]	NO DATA
<i>Pseudomonas aeruginosa</i>	PAO1	NO DATA	[26 33 39 24]	NO DATA
<i>Pseudomonas fluorescens</i>	Pf0-1	NO DATA	[26 33 34 29]	NO DATA
<i>Pseudomonas putida</i>	KT2440	NO DATA	[25 34 36 27]	NO DATA
<i>Legionella pneumophila</i>	Philadelphia-1	NO DATA	NO DATA	NO DATA
<i>Francisella tularensis</i>	schu 4	NO DATA	[33 32 25 32]	NO DATA
<i>Bordetella pertussis</i>	Tohama I	NO DATA	[26 33 39 24]	NO DATA
<i>Burkholderia cepacia</i>	J2315	NO DATA	[25 37 33 27]	NO DATA
<i>Burkholderia pseudomallei</i>	K96243	NO DATA	[25 37 34 26]	NO DATA
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	[17 23 22 10]	[29 31 32 30]	NO DATA
<i>Neisseria meningitidis</i>	MC58 (serogroup B)	NO DATA	[29 30 32 31]	NO DATA
<i>Neisseria meningitidis</i>	serogroup C, FAM18	NO DATA	[29 30 32 31]	NO DATA

<i>Neisseria meningitidis</i>	EZ491 (serogroup A)	NO DATA	[29 30 32 31]	NO DATA
<i>Chlamydomonas pneumoniae</i>	TW-183	NO DATA	NO DATA	NO DATA
<i>Chlamydomonas pneumoniae</i>	AR39	NO DATA	NO DATA	NO DATA
<i>Chlamydomonas pneumoniae</i>	CWL029	NO DATA	NO DATA	NO DATA
<i>Chlamydomonas pneumoniae</i>	J138	NO DATA	NO DATA	NO DATA
<i>Corynebacterium diphtheriae</i>	NCTC13129	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	k10	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	104	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	CSU#93	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	CDC 1551	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	NO DATA	NO DATA	NO DATA
<i>Mycoplasma pneumoniae</i>	M129	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MRSAA252	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	MSSA476	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	COL	[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
<i>Staphylococcus aureus</i>	Mu50	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	MW2	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	N315	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	NCTC 8325	[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
<i>Streptococcus agalactiae</i>	NEM316	[22 20 19 14]	[26 31 27 38]	[29 26 22 28]
<i>Streptococcus equi</i>	NC 002955	[22 21 19 13]	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS8232	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	MGAS315	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	SSI-1	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	MGAS10394	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	Manfredo (M5)	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	SF370 (M1)	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pneumoniae</i>	670	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
<i>Streptococcus pneumoniae</i>	R5	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
<i>Streptococcus pneumoniae</i>	TIGRA	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
<i>Streptococcus gordonii</i>	NCTC7868	[21 21 19 14]	NO DATA	[29 26 22 28]
<i>Streptococcus mitis</i>	NCTC 12261	[22 20 19 14]	[26 30 32 34]	NO DATA
<i>Streptococcus mutans</i>	UA159	NO DATA	NO DATA	NO DATA

Table 7D – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 355, 358, and 359

Organism	Strain	Primer 355 [A G C T]	Primer 358 [A G C T]	Primer 359 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	NO DATA	[24 39 33 20]	[25 21 24 17]
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	NO DATA	[26 34 35 21]	[23 23 19 22]
<i>Yersinia pestis</i>	KHS P12 (Biovar Mediævalis)	NO DATA	[26 34 35 21]	[23 23 19 22]
<i>Yersinia pestis</i>	91001	NO DATA	[26 34 35 21]	[23 23 19 22]
<i>Haemophilus influenzae</i>	KN20	NO DATA	NO DATA	NO DATA
<i>Pseudomonas aeruginosa</i>	PAO1	NO DATA	NO DATA	NO DATA
<i>Pseudomonas fluorescens</i>	Pf0-1	NO DATA	NO DATA	NO DATA
<i>Pseudomonas putida</i>	KT2440	NO DATA	[21 37 37 21]	NO DATA
<i>Legionella pneumophila</i>	Philadelphia-1	NO DATA	NO DATA	NO DATA
<i>Francisella tularensis</i>	schu 4	NO DATA	NO DATA	NO DATA
<i>Bordetella pertussis</i>	Tohama I	NO DATA	NO DATA	NO DATA
<i>Burkholderia cepacia</i>	J2315	NO DATA	NO DATA	NO DATA
<i>Burkholderia pseudomallei</i>	K96243	NO DATA	NO DATA	NO DATA
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	MCS9 (serogroup B)	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	serogroup C, FAM18	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	Z2491 (serogroup A)	NO DATA	NO DATA	NO DATA
<i>Chlamydia pneumoniae</i>	TW-183	NO DATA	NO DATA	NO DATA
<i>Chlamydia pneumoniae</i>	AR39	NO DATA	NO DATA	NO DATA
<i>Chlamydia pneumoniae</i>	CWL029	NO DATA	NO DATA	NO DATA
<i>Chlamydia pneumoniae</i>	J138	NO DATA	NO DATA	NO DATA
<i>Corynebacterium diphtheriae</i>	NCTC13129	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	k10	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	104	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	CSU#93	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	CDC 1551	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	NO DATA	NO DATA	NO DATA
<i>Mycoplasma pneumoniae</i>	M129	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MSSA476	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	COL	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	Mu50	NO DATA	NO DATA	NO DATA

<i>Staphylococcus aureus</i>	MF2	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	N315	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	NCTC 8325	NO DATA	NO DATA	NO DATA
<i>Streptococcus agalactiae</i>	NEM316	NO DATA	NO DATA	NO DATA
<i>Streptococcus equi</i>	NC 002955	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS8232	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS315	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	SSI-1	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS10394	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	Manfredo (MS)	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	SP370 (M1)	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	670	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	R6	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	TIGR4	NO DATA	NO DATA	NO DATA
<i>Streptococcus gordonii</i>	NCTC7868	NO DATA	NO DATA	NO DATA
<i>Streptococcus mitis</i>	NCTC 12261	NO DATA	NO DATA	NO DATA
<i>Streptococcus mutans</i>	UA159	NO DATA	NO DATA	NO DATA

Table 7E – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 362, 363, and 367

Organism	Strain	Primer 362 [A G C T]	Primer 363 [A G C T]	Primer 367 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	[21 33 22 16]	[16 34 26 26]	NO DATA
<i>Yersinia pestis</i>	CO-92 Biovar orientalis	[20 34 18 20]	NO DATA	NO DATA
<i>Yersinia pestis</i>	KIM5 P12 (Biovar Mediaevalis)	[20 34 18 20]	NO DATA	NO DATA
<i>Yersinia pestis</i>	91001	[20 34 18 20]	NO DATA	NO DATA
<i>Haemophilus influenzae</i>	KW20	NO DATA	NO DATA	NO DATA
<i>Pseudomonas aeruginosa</i>	PAO1	[19 35 21 17]	[16 36 28 22]	NO DATA
<i>Pseudomonas fluorescens</i>	PF0-1	NO DATA	[18 35 26 23]	NO DATA
<i>Pseudomonas putida</i>	KT2440	NO DATA	[16 35 28 23]	NO DATA
<i>Legionella pneumophila</i>	Philadelphia-1	NO DATA	NO DATA	NO DATA
<i>Francisella tularensis</i>	schu 4	NO DATA	NO DATA	NO DATA
<i>Bordetella pertussis</i>	Tohama I	[20 31 24 17]	[15 34 32 21]	[26 25 34 19]
<i>Burkholderia cepacia</i>	J2315	[20 33 21 18]	[15 36 26 25]	[25 27 32 20]
<i>Burkholderia pseudomallei</i>	K96243	[19 34 19 20]	[15 37 28 22]	[25 27 32 20]
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	NO DATA	NO DATA	NO DATA

<i>Neisseria meningitidis</i>	MCS8 (serogroup B)	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	serogroup C, FAM18	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	E2491 (serogroup A)	NO DATA	NO DATA	NO DATA
<i>Chlamydomonas pneumoniae</i>	TW-183	NO DATA	NO DATA	NO DATA
<i>Chlamydomonas pneumoniae</i>	AR39	NO DATA	NO DATA	NO DATA
<i>Chlamydomonas pneumoniae</i>	CWL029	NO DATA	NO DATA	NO DATA
<i>Chlamydomonas pneumoniae</i>	J138	NO DATA	NO DATA	NO DATA
<i>Corynebacterium diphtheriae</i>	NCTC13129	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	K10	[19 34 23 16]	NO DATA	[24 26 35 19]
<i>Mycobacterium avium</i>	104	[19 34 23 16]	NO DATA	[24 26 35 19]
<i>Mycobacterium tuberculosis</i>	CSU#93	[19 31 25 17]	NO DATA	[25 25 34 20]
<i>Mycobacterium tuberculosis</i>	CDC 1551	[19 31 24 18]	NO DATA	[25 25 34 20]
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	[19 31 24 18]	NO DATA	[25 25 34 20]
<i>Mycoplasma pneumoniae</i>	M129	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MSSA476	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	COL	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	Mu50	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MW2	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	N315	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	NCTC 8325	NO DATA	NO DATA	NO DATA
<i>Streptococcus agalactiae</i>	NEM316	NO DATA	NO DATA	NO DATA
<i>Streptococcus equi</i>	NC 002955	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS8232	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS315	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	SSI-1	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS10394	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	Manfredo (M5)	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	SF370 (M1)	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	670	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	R6	[20 30 19 23]	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	TIGR4	[20 30 19 23]	NO DATA	NO DATA
<i>Streptococcus gordonii</i>	NCTC7868	NO DATA	NO DATA	NO DATA
<i>Streptococcus mitis</i>	NCTC 12261	NO DATA	NO DATA	NO DATA
<i>Streptococcus</i>	UA159	NO DATA	NO DATA	NO DATA

<i>mutans</i>				
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[396] Four sets of throat samples from military recruits at different military facilities taken at different time points were analyzed using the primers of the present invention. The first set was collected at a military training center from November 1 to December 20, 2002 during one of the most severe outbreaks of pneumonia associated with group A *Streptococcus* in the United States since 1968. During this outbreak, fifty-one throat swabs were taken from both healthy and hospitalized recruits and plated on blood agar for selection of putative group A *Streptococcus* colonies. A second set of 15 original patient specimens was taken during the height of this group A *Streptococcus* -associated respiratory disease outbreak. The third set were historical samples, including twenty-seven isolates of group A *Streptococcus*, from disease outbreaks at this and other military training facilities during previous years. The fourth set of samples was collected from five geographically separated military facilities in the continental U.S. in the winter immediately following the severe November/December 2002 outbreak.

[397] Pure colonies isolated from group A *Streptococcus*-selective media from all four collection periods were analyzed with the surveillance primer set. All samples showed base compositions that precisely matched the four completely sequenced strains of *Streptococcus pyogenes*. Shown in Figure 4 is a 3D diagram of base composition (axes A, G and C) of bioagent identifying amplicons obtained with primer pair number 14 (a precursor of primer pair number 348 which targets 16S rRNA). The diagram indicates that the experimentally determined base compositions of the clinical samples closely match the base compositions expected for *Streptococcus pyogenes* and are distinct from the expected base compositions of other organisms.

[398] In addition to the identification of *Streptococcus pyogenes*, other potentially pathogenic organisms were identified concurrently. Mass spectral analysis of a sample whose nucleic acid was amplified by primer pair number 349 (SEQ ID NOs: 401:1156) exhibited signals of bioagent identifying amplicons with molecular masses that were found to correspond to analogous base compositions of bioagent identifying amplicons of *Streptococcus pyogenes* (A27 G32 C24 T18), *Neisseria meningitidis* (A25 G27 C22 T18), and *Haemophilus influenzae* (A28 G28 C25 T20) (see Figure 5 and Table 7B). These organisms were present in a ratio of 4:5:20 as determined by comparison of peak heights with peak height of an internal PCR calibration standard as described in commonly owned U.S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[399] Since certain division-wide primers that target housekeeping genes are designed to provide coverage of specific divisions of bacteria to increase the confidence level for identification of bacterial species, they are not expected to yield bioagent identifying amplicons for organisms outside of the specific divisions. For example, primer pair number 356 (SEQ ID NOs: 449:1380) primarily amplifies the nucleic acid of members of the classes *Bacilli* and *Clostridia* and is not expected to amplify proteobacteria such as *Neisseria meningitidis* and *Haemophilus influenzae*. As expected, analysis of the mass spectrum of amplification products obtained with primer pair number 356 does not indicate the presence of *Neisseria meningitidis* and *Haemophilus influenzae* but does indicate the presence of *Streptococcus pyogenes* (Figures 3 and 6, Table 7B). Thus, these primers or types of primers can confirm the absence of particular bioagents from a sample.

[400] The 15 throat swabs from military recruits were found to contain a relatively small set of microbes in high abundance. The most common were *Haemophilus influenza*, *Neisseria meningitidis*, and *Streptococcus pyogenes*. *Staphylococcus epidermidis*, *Moraxella catarrhalis*, *Corynebacterium pseudodiphtheriticum*, and *Staphylococcus aureus* were present in fewer samples. An equal number of samples from healthy volunteers from three different geographic locations, were identically analyzed. Results indicated that the healthy volunteers have bacterial flora dominated by multiple, commensal non-beta-hemolytic *Streptococcal* species, including the viridans group *streptococci* (*S. parasanguis*, *S. vestibularis*, *S. mitis*, *S. oralis* and *S. pneumoniae*; data not shown), and none of the organisms found in the military recruits were found in the healthy controls at concentrations detectable by mass spectrometry. Thus, the military recruits in the midst of a respiratory disease outbreak had a dramatically different microbial population than that experienced by the general population in the absence of epidemic disease.

Example 7: Triangulation Genotyping Analysis for Determination of emm-Type of *Streptococcus pyogenes* in Epidemic Surveillance

[401] As a continuation of the epidemic surveillance investigation of Example 6, determination of sub-species characteristics (genotyping) of *Streptococcus pyogenes*, was carried out based on a strategy that generates strain-specific signatures according to the rationale of Multi-Locus Sequence Typing (MLST). In classic MLST analysis, internal fragments of several housekeeping genes are amplified and sequenced (Enright et al. *Infection and Immunity*, 2001, 69, 2416-2427). In classic MLST analysis, internal fragments of several housekeeping genes are amplified and sequenced. In the present investigation, bioagent identifying amplicons from housekeeping genes were produced using drill-down primers and analyzed by mass spectrometry. Since mass spectral analysis results in molecular mass,

from which base composition can be determined, the challenge was to determine whether resolution of *emm* classification of strains of *Streptococcus pyogenes* could be determined.

[402] For the purpose of development of a triangulation genotyping assay, an alignment was constructed of concatenated alleles of seven MLST housekeeping genes (glucose kinase (*gki*), glutamine transporter protein (*gtr*), glutamate racemase (*murI*), DNA mismatch repair protein (*mutS*), xanthine phosphoribosyl transferase (*xpt*), and acetyl-CoA acetyl transferase (*yqiL*)) from each of the 212 previously *emm*-typed strains of *Streptococcus pyogenes*. From this alignment, the number and location of primer pairs that would maximize strain identification via base composition was determined. As a result, 6 primer pairs were chosen as standard drill-down primers for determination of *emm*-type of *Streptococcus pyogenes*. These six primer pairs are displayed in Table 8. This drill-down set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row.

Table 8: Triangulation Genotyping Analysis Primer Pairs for Group A *Streptococcus* Drill-Down

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
442	SP101_SPET11_358_387_TMOD_F	588	SP101_SPET11_448_473_TMOD_R	998	<i>gki</i>
80	SP101_SPET11_358_387_F	126	SP101_SPET11_448_473_TMOD_R	766	<i>gki</i>
443	SP101_SPET11_600_629_TMOD_F	348	SP101_SPET11_686_714_TMOD_R	1018	<i>gtr</i>
81	SP101_SPET11_600_629_F	62	SP101_SPET11_686_714_R	772	<i>gtr</i>
426	SP101_SPET11_1314_1336_TMOD_F	363	SP101_SPET11_1403_1431_TMOD_R	849	<i>murI</i>
86	SP101_SPET11_1314_1336_F	68	SP101_SPET11_1403_1431_R	711	<i>murI</i>
430	SP101_SPET11_1807_1835_TMOD_F	235	SP101_SPET11_1901_1927_TMOD_R	1439	<i>mutS</i>
90	SP101_SPET11_1807_1835_F	33	SP101_SPET11_1901_1927_R	1412	<i>mutS</i>
438	SP101_SPET11_3075_3103_TMOD_F	473	SP101_SPET11_3168_3196_TMOD_R	875	<i>xpt</i>
96	SP101_SPET11_3075_3103_F	108	SP101_SPET11_3168_3196_R	715	<i>xpt</i>
441	SP101_SPET11_3511_3535_TMOD_F	531	SP101_SPET11_3605_3629_TMOD_R	1294	<i>yqiL</i>
98	SP101_SPET11_3511_3535_F	116	SP101_SPET11_3605_3629_R	832	<i>yqiL</i>

[403] The primers of Table 8 were used to produce bioagent identifying amplicons from nucleic acid present in the clinical samples. The bioagent identifying amplicons which were subsequently analyzed by mass spectrometry and base compositions corresponding to the molecular masses were calculated.

[404] Of the 51 samples taken during the peak of the November/December 2002 epidemic (Table 9A-C rows 1-3), all except three samples were found to represent *emm3*, a Group A *Streptococcus* genotype previously associated with high respiratory virulence. The three outliers were from samples obtained from healthy individuals and probably represent non-epidemic strains. Archived samples (Tables 9A-C, rows 5-13) from historical collections showed a greater heterogeneity of base compositions and *emm* types as would be expected from different epidemics occurring at different places and dates. The results of the mass spectrometry analysis and *emm* gene sequencing were found to be concordant for the epidemic and historical samples.

Table 9A: Base Composition Analysis of Bioagent Identifying Amplicons of Group A *Streptococcus* samples from Six Military Installations Obtained with Primer Pair Nos. 426 and 430

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	murI (Primer Pair No. 426)	mutS (Primer Pair No. 430)
48	3	3	MCRD San Diego (Cultured)	2002	A39 G25 C20 T34	A38 G27 C23 T33
2	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	28	28			A39 G25 C20 T34	A38 G27 C23 T33
15	3	ND			A39 G25 C20 T34	A38 G27 C23 T33
6	3	3			A39 G25 C20 T34	A38 G27 C23 T33
3	5,58	5	NHRC San Diego-Archive (Cultured)	2003	A40 G24 C20 T34	A38 G27 C23 T33
6	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	11	11			A39 G25 C20 T34	A38 G27 C23 T33
3	12	12			A40 G24 C20 T34	A38 G26 C24 T33
1	22	22			A39 G25 C20 T34	A38 G27 C23 T33
3	25,75	75	Ft. Leonard Wood (Cultured)	2003	A39 G25 C20 T34	A38 G27 C23 T33
4	44/61,82,9	44/61			A40 G24 C20 T34	A38 G26 C24 T33
2	53,91	91			A39 G25 C20 T34	A38 G27 C23 T33
1	2	2			A39 G25 C20 T34	A38 G27 C24 T32
2	3	3			A39 G25 C20 T34	A38 G27 C23 T32
1	4	4	Ft. Sill (Cultured)	2003	A39 G25 C20 T34	A38 G27 C23 T33
1	6	6			A40 G24 C20 T34	A38 G27 C23 T33
11	25 or 75	75			A39 G25 C20 T34	A38 G27 C23 T33
1	25,75, 33, 34,4,52,84	75			A39 G25 C20 T34	A38 G27 C23 T33
1	44/61 or 82 or 9	44/61			A40 G24 C20 T34	A38 G26 C24 T33
2	5 or 58	5	Ft. Sill (Cultured)	2003	A40 G24 C20 T34	A38 G27 C23 T33
3	1	1			A40 G24 C20 T34	A38 G27 C23 T33
2	3	3			A39 G25 C20 T34	A38 G27 C23 T33
1	4	4			A39 G25 C20 T34	A38 G27 C23 T33
1	28	28			A39 G25 C20 T34	A38 G27 C23 T33
1	3	3	Ft.	2003	A39 G25 C20 T34	A38 G27 C23 T33

1	4	4	Benning (Cultured)		A39 G25 C20 T34	A38 G27 C23 T33
3	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	11	11			A39 G25 C20 T34	A38 G27 C23 T33
1	13	94**			A40 G24 C20 T34	A38 G27 C23 T33
1	44/61 or 82 or 9	82	Lackland AFB (Throat Swabs)	2003	A40 G24 C20 T34	A38 G26 C24 T33
1	5 or 58	58			A40 G24 C20 T34	A38 G27 C23 T33
1	78 or 89	89			A39 G25 C20 T34	A38 G27 C23 T33
2	5 or 58				A40 G24 C20 T34	A38 G27 C23 T33
1	2				A39 G25 C20 T34	A38 G27 C24 T32
1	81 or 90	ND			A40 G24 C20 T34	A38 G27 C23 T33
1	78				A38 G26 C20 T34	A38 G27 C23 T33
3***	No detection				No detection	No detection
7	3	ND			A39 G25 C20 T34	A38 G27 C23 T33
1	3	ND			No detection	A38 G27 C23 T33
1	3	ND	MCRD San Diego (Throat Swabs)	2002	No detection	No detection
1	3	ND			No detection	No detection
2	3	ND			No detection	A38 G27 C23 T33
3	No detection	ND			No detection	No detection

**Table 9B: Base Composition Analysis of Bioagent Identifying Amplicons of Group A
Streptococcus samples from Six Military Installations Obtained with Primer Pair Nos. 438 and 441**

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	xpt (Primer Pair No. 438)	yqilL (Primer Pair No. 441)
48	3	3	MCRD San Diego (Cultured)	2002	A30 G36 C20 T36	A40 G29 C19 T31
2	6	6			A30 G36 C20 T36	A40 G29 C19 T31
1	28	28			A30 G36 C20 T36	A41 G28 C18 T32
15	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
6	3	3	NHRC San Diego- Archive (Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
3	5,58	5			A30 G36 C20 T36	A40 G29 C19 T31
6	6	6			A30 G36 C20 T36	A40 G29 C19 T31
1	11	11			A30 G36 C20 T36	A40 G29 C19 T31
3	12	12			A30 G36 C19 T37	A40 G29 C19 T31
1	22	22			A30 G36 C20 T36	A40 G29 C19 T31
3	25,75	75			A30 G36 C20 T36	A40 G29 C19 T31
4	44/61,82,9	44/61			A30 G36 C20 T36	A41 G28 C19 T31
2	53,91	91			A30 G36 C19 T37	A40 G29 C19 T31
1	2	2			A30 G36 C20 T36	A40 G29 C19 T31
2	3	3	Ft. Leonard Wood (Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31
1	6	6			A30 G36 C20 T36	A40 G29 C19 T31
11	25 or 75	75			A30 G36 C20 T36	A40 G29 C19 T31
1	25,75, 33, 34,4,52,84	75	Ft. Sill (Cultured)	2003	A30 G36 C19 T37	A40 G29 C19 T31
1	44/61 or 82 or 9	44/61			A30 G36 C20 T36	A41 G28 C19 T31
2	5 or 58	5			A30 G36 C20 T36	A40 G29 C19 T31
3	1	1			A30 G36 C19 T37	A40 G29 C19 T31
2	3	3	(Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31

1	28	28	Ft. Benning (Cultured)	2003	A30 G36 C20 T36	A41 G28 C18 T32
1	3	3			A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31
3	6	6			A30 G36 C20 T36	A40 G29 C19 T31
1	11	11			A30 G36 C20 T36	A40 G29 C19 T31
1	13	94**			A30 G36 C20 T36	A41 G28 C19 T31
1	44/61 or 82 or 9	82			A30 G36 C20 T36	A41 G28 C19 T31
1	5 or 58	58			A30 G36 C20 T36	A40 G29 C19 T31
1	78 or 89	89			A30 G36 C20 T36	A41 G28 C19 T31
2	5 or 58				A30 G36 C20 T36	A40 G29 C19 T31
1	2	ND	Lackland AFB (Throat Swabs)	2003	A30 G36 C20 T36	A40 G29 C19 T31
1	81 or 90				A30 G36 C20 T36	A40 G29 C19 T31
1	78				A30 G36 C20 T36	A41 G28 C19 T31
3***	No detection				No detection	No detection
7	3	ND	MCRD San Diego (Throat Swabs)	2002	A30 G36 C20 T36	A40 G29 C19 T31
1	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
1	3	ND			A30 G36 C20 T36	No detection
1	3	ND			No detection	A40 G29 C19 T31
2	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
3	No detection	ND			No detection	No detection

Table 9C: Base Composition Analysis of Bioagent Identifying Amplicons of Group A *Streptococcus* samples from Six Military Installations Obtained with Primer Pair Nos. 438 and 441

# of Instances	emm-type by Mass Spectrometry	emm-Genes Sequencing	Location (sample)	Year	gk1 (Primer Pair No. 442)	gtr ((Primer Pair No. 443)
48	3	3	MCRD San Diego (Cultured)	2002	A32 G35 C17 T32	A39 G28 C16 T32
2	6	6			A31 G35 C17 T33	A39 G28 C15 T33
1	28	28			A30 G36 C17 T33	A39 G28 C16 T32
15	3	ND			A32 G35 C17 T32	A39 G28 C16 T32
6	3	3			A32 G35 C17 T32	A39 G28 C16 T32
3	5,58	5	NHRC San Diego-Archive (Cultured)	2003	A30 G36 C20 T30	A39 G28 C15 T33
6	6	6			A31 G35 C17 T33	A39 G28 C15 T33
1	11	11			A30 G36 C20 T30	A39 G28 C16 T32
3	12	12			A31 G35 C17 T33	A39 G28 C15 T33
1	22	22			A31 G35 C17 T33	A38 G29 C15 T33
3	25,75	75			A30 G36 C17 T33	A39 G28 C15 T32
4	44/61,82,9	44/61			A30 G36 C18 T32	A39 G28 C15 T33
2	53,91	91			A32 G35 C17 T32	A39 G28 C16 T32
1	2	2			A30 G36 C17 T33	A39 G28 C15 T33
2	3	3			A32 G35 C17 T32	A39 G28 C16 T32
1	4	4	Ft. Leonard Wood (Cultured)	2003	A31 G35 C17 T33	A39 G28 C15 T33
1	6	6			A31 G35 C17 T33	A39 G28 C15 T33
11	25 or 75	75			A30 G36 C17 T33	A39 G28 C15 T33
1	25,75, 33, 34,4,52,84	75			A30 G36 C17 T33	A39 G28 C15 T33
1	44/61 or 82 or 9	44/61			A30 G36 C18 T32	A39 G28 C15 T33
2	5 or 58	5	Ft. Sill	2003	A30 G36 C20 T30	A39 G28 C15 T33
3	1	1			A30 G36 C18 T32	A39 G28 C15 T33
2	3	3			A32 G35 C17 T32	A39 G28 C16 T32

1	4	4	(Cultured)		A31 G35 C17 T33	A39 G28 C15 T33
1	28	28			A30 G36 C17 T33	A39 G28 C16 T32
1	3	3			A32 G35 C17 T32	A39 G28 C16 T32
1	4	4			A31 G35 C17 T33	A39 G28 C15 T33
3	6	6			A31 G35 C17 T33	A39 G28 C15 T33
1	11	11			A30 G36 C20 T30	A39 G28 C16 T32
1	13	94**			A30 G36 C19 T31	A39 G28 C15 T33
1	44/61 or 82 or 9	82			A30 G36 C18 T32	A39 G28 C15 T33
1	5 or 58	58			A30 G36 C20 T30	A39 G28 C15 T33
1	78 or 89	89			A30 G36 C18 T32	A39 G28 C15 T33
2	5 or 58				A30 G36 C20 T30	A39 G28 C15 T33
1	2				A30 G36 C17 T33	A39 G28 C15 T33
1	81 or 90	ND			A30 G36 C17 T33	A39 G28 C15 T33
1	78				A30 G36 C18 T32	A39 G28 C15 T33
3***	No detection				No detection	No detection
7	3	ND			A32 G35 C17 T32	A39 G28 C16 T32
1	3	ND			No detection	No detection
1	3	ND			A32 G35 C17 T32	A39 G28 C16 T32
1	3	ND			A32 G35 C17 T32	No detection
2	3	ND			A32 G35 C17 T32	No detection
3	No detection	ND			No detection	No detection

Example 8: Design of Calibrant Polynucleotides based on Bioagent Identifying Amplicons for Identification of Species of Bacteria (Bacterial Bioagent Identifying Amplicons)

[405] This example describes the design of 19 calibrant polynucleotides based on bacterial bioagent identifying amplicons corresponding to the primers of the broad surveillance set (Table 5) and the *Bacillus anthracis* drill-down set (Table 6).

[406] Calibration sequences were designed to simulate bacterial bioagent identifying amplicons produced by the T modified primer pairs shown in Tables 5 and 6 (primer names have the designation "TMOD"). The calibration sequences were chosen as a representative member of the section of bacterial genome from specific bacterial species which would be amplified by a given primer pair. The model bacterial species upon which the calibration sequences are based are also shown in Table 10. For example, the calibration sequence chosen to correspond to an amplicon produced by primer pair no. 361 is SEQ ID NO: 1445. In Table 10, the forward (_F) or reverse (_R) primer name indicates the coordinates of an extraction representing a gene of a standard reference bacterial genome to which the primer hybridizes e.g.: the forward primer name 16S_EC_713_732_TMOD_F indicates that the forward primer hybridizes to residues 713-732 of the gene encoding 16S ribosomal RNA in an *E. coli* reference sequence (in this case, the reference sequence is an extraction consisting of residues 4033120-4034661 of the genomic sequence of *E. coli* K12 (GenBank gi number 16127994). Additional gene coordinate reference information is shown in Table 11. The designation "TMOD" in the primer names indicates that the 5' end of the primer has been modified with a non-matched template T residue which

prevents the PCR polymerase from adding non-templated adenosine residues to the 5' end of the amplification product, an occurrence which may result in miscalculation of base composition from molecular mass data (*vide supra*).

[0143] The 19 calibration sequences described in Tables 10 and 11 were combined into a single calibration polynucleotide sequence (SEQ ID NO: 1464 - which is herein designated a "combination calibration polynucleotide") which was then cloned into a pCR®-Blunt vector (Invitrogen, Carlsbad, CA). This combination calibration polynucleotide can be used in conjunction with the primers of Tables 5 or 6 as an internal standard to produce calibration amplicons for use in determination of the quantity of any bacterial bioagent. Thus, for example, when the combination calibration polynucleotide vector is present in an amplification reaction mixture, a calibration amplicon based on primer pair 346 (16S rRNA) will be produced in an amplification reaction with primer pair 346 and a calibration amplicon based on primer pair 363 (rpoC) will be produced with primer pair 363. Coordinates of each of the 19 calibration sequences within the calibration polynucleotide (SEQ ID NO: 1464) are indicated in Table 11.

Table 10: Bacterial Primer Pairs for Production of Bacterial Bioagent Identifying Amplicons and Corresponding Representative Calibration Sequences

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO)	Reverse Primer Name	Reverse Primer (SEQ ID NO)	Calibration Sequence Model Species	Calibration Sequence (SEQ ID NO)
361	16S_EC_1090_1111_2_TMOD_F	697	16S_EC_1175_1196_TMOD_R	1398	<i>Bacillus anthracis</i>	1445
346	16S_EC_713_732_TMOD_F	202	16S_EC_789_809_TMOD_R	1110	<i>Bacillus anthracis</i>	1446
347	16S_EC_785_806_TMOD_F	560	16S_EC_880_897_TMOD_R	1278	<i>Bacillus anthracis</i>	1447
348	16S_EC_960_981_TMOD_F	706	16S_EC_1054_1073_TMOD_R	895	<i>Bacillus anthracis</i>	1448
349	23S_EC_1826_1843_TMOD_F	401	23S_EC_1906_1924_TMOD_R	1156	<i>Bacillus anthracis</i>	1449
360	23S_EC_2646_2667_TMOD_F	409	23S_EC_2745_2765_TMOD_R	1434	<i>Bacillus anthracis</i>	1450
350	CAPC_BA_274_303_TMOD_F	476	CAPC_BA_349_376_TMOD_R	1314	<i>Bacillus anthracis</i>	1451
351	CYA_BA_1353_1379_TMOD_F	355	CYA_BA_1448_1467_TMOD_R	1423	<i>Bacillus anthracis</i>	1452
352	INF8_EC_1365_1393_TM_OD_F	687	INF8_EC_1439_1467_TMOD_R	1411	<i>Bacillus anthracis</i>	1453
353	LEF_BA_756_781_TMOD_F	220	LEF_BA_843_872_TMOD_R	1394	<i>Bacillus anthracis</i>	1454
356	RPLB_EC_650_679_TMOD_F	449	RPLB_EC_739_762_TMOD_R	1380	<i>Clostridium botulinum</i>	1455
449	RPLB_EC_680_710_F	309	RPLB_EC_737_758_R	1336	<i>Clostridium botulinum</i>	1456
359	RPOB_EC_1845_1866_TM_OD_F	659	RPOB_EC_1909_1929_TMOD_R	1250	<i>Yersinia Pestis</i>	1457
362	RPOB_EC_3759_3821_TM_OD_F	581	RPOB_EC_3862_3888_TMOD_R	1325	<i>Burkholderia mallei</i>	1458
363	RPOC_EC_2146_2174_TM_OD_F	284	RPOC_EC_2227_2245_TMOD_R	898	<i>Burkholderia mallei</i>	1459

354	RPOC_EC_2218_2241_TM OD_F	405	RPOC_EC_2313_2337_TM OD_R	1072	<i>Bacillus anthracis</i>	1460
355	SSPE_BA_115_137_TM OD_F	255	SSPE_BA_197_222_TM OD_R	1402	<i>Bacillus anthracis</i>	1461
367	TUFB_EC_957_979_TM OD_F	308	TUFB_EC_1034_1058_TM OD_R	1276	<i>Sarkoidia mallei</i>	1462
358	VALS_EC_1105_1124_TM OD_F	385	VALS_EC_1195_1218_TM OD_R	1093	<i>Yersinia Pestis</i>	1463

Table 11: Primer Pair Gene Coordinate References and Calibration Polynucleotide Sequence
Coordinates within the Combination Calibration Polynucleotide

Bacterial Gene and Species	Gene Extraction Coordinates of Genomic (G) or Plasmid (P) Sequence	Reference GenBank GI No. of Genomic (G) or Plasmid (P) Sequence	Primer Pair No.	Coordinates of Calibration Sequence in Combination Calibration polynucleotide (SEQ ID NO: 1464)
16S <i>E. coli</i>	4033120..4034661	16127994 (G)	346	16..109
16S <i>E. coli</i>	4033120..4034661	16127994 (G)	347	83..190
16S <i>E. coli</i>	4033120..4034661	16127994 (G)	348	246..353
23S <i>E. coli</i>	4166220..4169123	16127994 (G)	361	368..469
23S <i>E. coli</i>	4166220..4169123	16127994 (G)	349	743..837
23S <i>E. coli</i>	4166220..4169123	16127994 (G)	360	865..981
rcpB <i>E. coli</i>	4178823..4182851 (complement strand)	16127994 (G)	359	1591..1672
rcpB <i>E. coli</i>	4178823..4182851 (complement strand)	16127994 (G)	362	2081..2167
rcpC <i>E. coli</i>	4182928..4187151	16127994 (G)	354	1810..1926
rcpC <i>E. coli</i>	4182928..4187151	16127994 (G)	363	2183..2279
infB <i>E. coli</i>	3311655..3310983 (complement strand)	16127994 (G)	352	1692..1751
tuftB <i>E. coli</i>	4173523..4174707	16127994 (G)	367	2400..2498
rp1B <i>E. coli</i>	3449001..3448180	16127994 (G)	356	1945..2060
rp1B <i>E. coli</i>	3449001..3448180	16127994 (G)	449	1986..2055
valS <i>E. coli</i>	4481405..4478550 (complement strand)	16127994 (G)	358	1462..1572
capC <i>B. anthracis</i>	56074..55628 (complement strand)	6470151 (P)	350	2517..2616
cya <i>B. anthracis</i>	156626..154288 (complement strand)	4894216 (P)	351	1338..1449
lef <i>B. anthracis</i>	127442..129921	4894216 (P)	353	1321..1234
sepB <i>B. anthracis</i>	226496..226783	30253828 (P)	355	1007..1104

Example 9: Use of a Calibration Polynucleotide for Determining the Quantity of *Bacillus Anthracis* in a Sample Containing a Mixture of Microbes

[407] The process described in this example is shown in Figure 2. The capC gene is a gene involved in capsule synthesis which resides on the pX02 plasmid of *Bacillus anthracis*. Primer pair number 350 (see Tables 10 and 11) was designed to identify *Bacillus anthracis* via production of a bacterial bioagent identifying amplicon. Known quantities of the combination calibration polynucleotide vector described in Example 8 were added to amplification mixtures containing bacterial bioagent nucleic acid from a mixture of microbes which included the Ames strain of *Bacillus anthracis*. Upon amplification of the bacterial bioagent nucleic acid and the combination calibration polynucleotide vector with primer pair no. 350, bacterial bioagent identifying amplicons and calibration amplicons were obtained and characterized by mass spectrometry. A mass spectrum measured for the amplification reaction is shown in Figure 7. The molecular masses of the bioagent identifying amplicons provided the means for identification of the bioagent from which they were obtained (Ames strain of *Bacillus*

anthracis) and the molecular masses of the calibration amplicons provided the means for their identification as well. The relationship between the abundance (peak height) of the calibration amplicon signals and the bacterial bioagent identifying amplicon signals provides the means of calculation of the copies of the pX02 plasmid of the Ames strain of *Bacillus anthracis*. Methods of calculating quantities of molecules based on internal calibration procedures are well known to those of ordinary skill in the art.

[408] Averaging the results of 10 repetitions of the experiment described above, enabled a calculation that indicated that the quantity of Ames strain of *Bacillus anthracis* present in the sample corresponds to approximately 10 copies of pX02 plasmid.

Example 10: Triangulation Genotyping Analysis of *Campylobacter* Species

[409] A series of triangulation genotyping analysis primers were designed as described in Example 1 with the objective of identification of different strains of *Campylobacter jejuni*. The primers are listed in Table 12 with the designation "CJST_CJ." Housekeeping genes to which the primers hybridize and produce bioagent identifying amplicons include: tkt (transketolase), glyA (serine hydroxymethyltransferase), gltA (citrate synthase), aspA (aspartate ammonia lyase), glnA (glutamine synthase), pgm (phosphoglycerate mutase), and uncA (ATP synthetase alpha chain).

Table 12: *Campylobacter* Genotyping Primer Pairs

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
1053	CJST_CJ_1080_1110_F	681	CJST_CJ_1166_1198_R	1022	gltA
1047	CJST_CJ_584_616_F	315	CJST_CJ_663_692_R	1379	glnA
1048	CJST_CJ_360_394_F	346	CJST_CJ_442_476_R	955	aspA
1049	CJST_CJ_2636_2668_F	504	CJST_CJ_2753_2777_R	1409	tkt
1054	CJST_CJ_2060_2090_F	323	CJST_CJ_2148_2174_R	1068	pgm
1064	CJST_CJ_1680_1713_F	479	CJST_CJ_1795_1822_R	938	glyA

[410] The primers were used to amplify nucleic acid from 50 food product samples provided by the USDA, 25 of which contained *Campylobacter jejuni* and 25 of which contained *Campylobacter coli*. Primers used in this study were developed primarily for the discrimination of *Campylobacter jejuni* clonal complexes and for distinguishing *Campylobacter jejuni* from *Campylobacter coli*. Finer discrimination between *Campylobacter coli* types is also possible by using specific primers targeted to loci where closely-related *Campylobacter coli* isolates demonstrate polymorphisms between strains. The conclusions of the comparison of base composition analysis with sequence analysis are shown in Tables 13A-C.

Table 13A – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down
MLST Primer Pair Nos: 1048 and 1047

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1048 (ampH)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1047 (glnA)
J-1	<i>C. jejuni</i>	Goose	ST 690 /692/707/991	ST 991	RM3673	A30 G25 C16 T46	A47 G21 C16 T25
J-2	<i>C. jejuni</i>	Human	Complex 296/48/353	ST 356, complex 353	RM4192	A30 G25 C16 T46	A48 G21 C17 T23
J-3	<i>C. jejuni</i>	Human	Complex 354/179	ST 436	RM4194	A30 G25 C15 T47	A48 G21 C18 T22
J-4	<i>C. jejuni</i>	Human	Complex 257	ST 257, complex 257	RM4197	A30 G25 C16 T46	A48 G21 C18 T22
J-5	<i>C. jejuni</i>	Human	Complex 52	ST 52, complex 52	RM4277	A30 G25 C16 T46	A48 G21 C17 T23
J-6	<i>C. jejuni</i>	Human	Complex 443	ST 51, complex 443	RM4275	A30 G25 C15 T47	A48 G21 C17 T23
					RM4279	A30 G25 C15 T47	A48 G21 C17 T23
J-7	<i>C. jejuni</i>	Human	Complex 42	ST 604, complex 42	RM1064	A30 G25 C15 T47	A48 G21 C18 T22
J-8	<i>C. jejuni</i>	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A30 G25 C15 T47	A48 G21 C18 T22
J-9	<i>C. jejuni</i>	Human	Complex 45/203	ST 147, Complex 45	RM3203	A30 G25 C15 T47	A47 G21 C18 T23
	<i>C. jejuni</i>			ST 828	RM4103	A31 G27 C20 T39	A48 G21 C16 T24
C-1	<i>C. coli</i>	Human	Consistent with 74 closely related sequence types (none belong to a clonal complex)	ST 832	RM1169	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1056	RM1057	A31 G27 C20 T39	A48 G21 C16 T24
				ST 889	RM1166	A31 G27 C20 T39	A48 G21 C16 T24
				ST 829	RM1182	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1050	RM1518	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1051	RM1521	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1053	RM1523	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1055	RM1527	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1017	RM1529	A31 G27 C20 T39	A48 G21 C16 T24
				ST 860	RM1840	A31 G27 C20 T39	A48 G21 C16 T24
		ST 1063		RM2219	A31 G27 C20 T39	A48 G21 C16 T24	
		ST 1066		RM2241	A31 G27 C20 T39	A48 G21 C16 T24	
		ST 1067		RM2243	A31 G27 C20 T39	A48 G21 C16 T24	
		ST 1068		RM2439	A31 G27 C20 T39	A48 G21 C16 T24	
		ST 1016		RM3230	A31 G27 C20 T39	A48 G21 C16 T24	
		ST 1069		RM3231	A31 G27 C20 T39	A48 G21 C16 T24	
		ST 1061		RM1904	A31 G27 C20 T39	A48 G21 C16 T24	
		ST 825		RM1534	A31 G27 C20 T39	A48 G21 C16 T24	
		ST 901		RM1505	A31 G27 C20 T39	A48 G21 C16 T24	
		C-2		<i>C. coli</i>	Human	ST 895	ST 895
C-3	<i>C. coli</i>	Poultry	Consistent	ST 1064	RM2223	A31 G27 C20 T39	A48 G21 C16 T24

			with 63 closely related sequence types (none belong to a clonal complex)	ST 1082	RM1178	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1054	RM1525	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1049	RM1517	A31 G27 C20 T39	A48 G21 C16 T24
		Marmoset		ST 891	RM1531	A31 G27 C20 T39	A48 G21 C16 T24

Table 13B – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1053 and 1064

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1053 (gltA)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1064 (glyA)
J-1	<i>C. jejuni</i>	Goose	ST 690 /692/707/991	ST 991	RM3673	A24 G25 C23 T47	A40 G29 C29 T45
J-2	<i>C. jejuni</i>	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A24 G25 C23 T47	A40 G29 C29 T45
J-3	<i>C. jejuni</i>	Human	Complex 354/172	ST 436	RM4194	A24 G25 C23 T47	A40 G29 C29 T45
J-4	<i>C. jejuni</i>	Human	Complex 257	ST 257, complex 257	RM4197	A24 G25 C23 T47	A40 G29 C29 T45
J-5	<i>C. jejuni</i>	Human	Complex 52	ST 52, complex 52	RM4277	A24 G25 C23 T47	A39 G30 C26 T48
J-6	<i>C. jejuni</i>	Human	Complex 443	ST 51, complex 443	RM4275 RM4279	A24 G25 C23 T47	A39 G30 C28 T46 A39 G30 C28 T46
J-7	<i>C. jejuni</i>	Human	Complex 42	ST 604, complex 42	RM1864	A24 G25 C23 T47	A39 G30 C26 T48
J-8	<i>C. jejuni</i>	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A24 G25 C23 T47	A38 G31 C28 T46
J-9	<i>C. jejuni</i>	Human	Complex 45/283	ST 147, Complex 45	RM3203	A24 G25 C23 T47	A38 G31 C28 T46
C-1	<i>C. jejuni</i>	Human	Consistent with 74 closely related sequence types (none belong to a clonal complex)	ST 828	RM4183	A23 G24 C26 T46	A39 G30 C27 T47
	ST 832			RM1169	A23 G24 C26 T46	A39 G30 C27 T47	
	ST 1055			RM1857	A23 G24 C26 T46	A39 G30 C27 T47	
	ST 889			RM1166	A23 G24 C26 T46	A39 G30 C27 T47	
	ST 829			RM1182	A23 G24 C26 T46	A39 G30 C27 T47	
	ST 1050			RM1518	A23 G24 C26 T46	A39 G30 C27 T47	
	ST 1051			RM1521	A23 G24 C26 T46	A39 G30 C27 T47	
	ST 1053			RM1523	A23 G24 C26 T46	A39 G30 C27 T47	
	ST 1055			RM1527	A23 G24 C26 T46	A39 G30 C27 T47	
	ST 1017			RM1529	A23 G24 C26 T46	A39 G30 C27 T47	
	Poultry			ST 860	RM1840	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1063	RM2219	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1066	RM2241	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1067	RM2243	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1068	RM2439	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1016	RM3230	A23 G24 C26 T46	A39 G30 C27 T47
	Swine						

		Unknown		ST 1069	RM3231	A23 G24 C26 T46	NO DATA
				ST 1061	RM1904	A23 G24 C26 T46	A39 G30 C27 T47
				ST 825	RM1534	A23 G24 C26 T46	A39 G30 C27 T47
				ST 901	RM1505	A23 G24 C26 T46	A39 G30 C27 T47
C-2	C. coli	Human	ST 895	ST 895	RM1532	A23 G24 C26 T46	A39 G30 C27 T47
C-3	C. coli	Poultry	Consistent with 63 closely related sequence types (none belong to a clonal complex)	ST 1064	RM2223	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1082	RM1178	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1054	RM1525	A23 G24 C26 T47	A39 G30 C27 T47
				ST 1049	RM1517	A23 G24 C26 T46	A39 G30 C27 T47
		Marmoset		ST 891	RM1531	A23 G24 C26 T46	A39 G30 C27 T47

Table 13C – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1054 and 1049

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1054 (pgm)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1049 (tkt)
J-1	C. jejuni	Goose	ST 690 /692/707/991	ST 991	RM3673	A26 G33 C19 T38	A41 G28 C35 T38
J-2	C. jejuni	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A26 G33 C19 T37	A41 G28 C36 T37
J-3	C. jejuni	Human	Complex 354/179	ST 436	RM4194	A27 G32 C19 T37	A42 G28 C36 T36
J-4	C. jejuni	Human	Complex 257	ST 257, complex 257	RM4197	A27 G32 C19 T37	A41 G29 C35 T37
J-5	C. jejuni	Human	Complex 52	ST 52, complex 52	RM4277	A26 G33 C18 T38	A41 G28 C36 T37
J-6	C. jejuni	Human	Complex 443	ST 51, complex 443	RM4275	A27 G31 C19 T38	A41 G28 C36 T37
					RM4279	A27 G31 C19 T38	A41 G28 C36 T37
J-7	C. jejuni	Human	Complex 42	ST 604, complex 42	RM1864	A27 G32 C19 T37	A42 G28 C35 T37
J-8	C. jejuni	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A26 G33 C19 T37	A42 G28 C35 T37
J-9	C. jejuni	Human	Complex 45/283	ST 147, Complex 45	RM3203	A28 G31 C19 T37	A43 G28 C36 T35
C-1	C. coli	Human	Consistent with 74 closely related sequence types (none belong to a clonal complex)	ST 828	RM4183	A27 G30 C19 T39	A46 G28 C32 T36
				ST 832	RM1169	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1056	RM1857	A27 G30 C19 T39	A46 G28 C32 T36
				ST 889	RM1166	A27 G30 C19 T39	A46 G28 C32 T36
				ST 829	RM1182	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1050	RM1518	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1051	RM1521	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1053	RM1523	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1055	RM1527	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1017	RM1529	A27 G30 C19 T39	A46 G28 C32 T36

				ST 860	RM1840	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1063	RM2219	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1066	RM2241	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1067	RM2243	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1068	RM2439	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1016	RM3230	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1069	RM3231	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1061	RM1904	A27 G30 C19 T39	A46 G28 C32 T36
				ST 825	RM1534	A27 G30 C19 T39	A46 G28 C32 T36
				ST 901	RM1505	A27 G30 C19 T39	A46 G28 C32 T36
				ST 895	RM1532	A27 G30 C19 T39	A46 G29 C32 T36
				ST 1064	RM2223	A27 G30 C19 T39	A46 G29 C32 T36
ST 1082	RM1178	A27 G30 C19 T39	A46 G29 C32 T36				
ST 1054	RM1525	A27 G30 C19 T39	A46 G29 C32 T36				
ST 1049	RM1517	A27 G30 C19 T39	A46 G29 C32 T36				
ST 891	RM1531	A27 G30 C19 T39	A46 G29 C32 T36				
C-2	C. coli	Human	ST 895	RM1532	A27 G30 C19 T39	A46 G29 C32 T36	
C-3	C. coli	Poultry	Consistent with 63 closely related sequence types (none belong to a clonal complex)	ST 1064	RM2223	A27 G30 C19 T39	A46 G29 C32 T36
				ST 1082	RM1178	A27 G30 C19 T39	A46 G29 C32 T36
				ST 1054	RM1525	A27 G30 C19 T39	A46 G29 C32 T36
				ST 1049	RM1517	A27 G30 C19 T39	A46 G29 C32 T36
		Marmoset		ST 891	RM1531	A27 G30 C19 T39	A46 G29 C32 T36

[411] The base composition analysis method was successful in identification of 12 different strain groups. *Campylobacter jejuni* and *Campylobacter coli* are generally differentiated by all loci. Ten clearly differentiated *Campylobacter jejuni* isolates and 2 major *Campylobacter coli* groups were identified even though the primers were designed for strain typing of *Campylobacter jejuni*. One isolate (RM4183) which was designated as *Campylobacter jejuni* was found to group with *Campylobacter coli* and also appears to actually be *Campylobacter coli* by full MLST sequencing.

Example 11: Identification of *Acinetobacter baumannii* Using Broad Range Survey and Division-Wide Primers in Epidemiological Surveillance

[412] To test the capability of the broad range survey and division-wide primer sets of Table 5 in identification of *Acinetobacter* species, 183 clinical samples were obtained from individuals participating in, or in contact with individuals participating in Operation Iraqi Freedom (including US service personnel, US civilian patients at the Walter Reed Army Institute of Research (WRAIR), medical staff, Iraqi civilians and enemy prisoners. In addition, 34 environmental samples were obtained from hospitals in Iraq, Kuwait, Germany, the United States and the USNS Comfort, a hospital ship.

[413] Upon amplification of nucleic acid obtained from the clinical samples, primer pairs 346-349, 360, 361, 354, 362 and 363 (Table 5) all produced bacterial bioagent amplicons which identified *Acinetobacter baumannii* in 215 of 217 samples. The organism *Klebsiella pneumoniae* was identified in the remaining two samples. In addition, 14 different strain types (containing single nucleotide polymorphisms relative to a reference strain of *Acinetobacter baumannii*) were identified and assigned

arbitrary numbers from 1 to 14. Strain type 1 was found in 134 of the sample isolates and strains 3 and 7 were found in 46 and 9 of the isolates respectively.

[414] The epidemiology of strain type 7 of *Acinetobacter baumannii* was investigated. Strain 7 was found in 4 patients and 5 environmental samples (from field hospitals in Iraq and Kuwait). The index patient infected with strain 7 was a pre-war patient who had a traumatic amputation in March of 2003 and was treated at a Kuwaiti hospital. The patient was subsequently transferred to a hospital in Germany and then to WRAIR. Two other patients from Kuwait infected with strain 7 were found to be non-infectious and were not further monitored. The fourth patient was diagnosed with a strain 7 infection in September of 2003 at WRAIR. Since the fourth patient was not related involved in Operation Iraqi Freedom, it was inferred that the fourth patient was the subject of a nosocomial infection acquired at WRAIR as a result of the spread of strain 7 from the index patient.

[415] The epidemiology of strain type 3 of *Acinetobacter baumannii* was also investigated. Strain type 3 was found in 46 samples, all of which were from patients (US service members, Iraqi civilians and enemy prisoners) who were treated on the USNS Comfort hospital ship and subsequently returned to Iraq or Kuwait. The occurrence of strain type 3 in a single locale may provide evidence that at least some of the infections at that locale were a result of nosocomial infections.

[416] This example thus illustrates an embodiment of the present invention wherein the methods of analysis of bacterial bioagent identifying amplicons provide the means for epidemiological surveillance.

Example 12: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for *Acinetobacter baumannii*

[417] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, an additional 21 primer pairs were selected based on analysis of housekeeping genes of the genus *Acinetobacter*. Genes to which the drill-down triangulation genotyping analysis primers hybridize for production of bacterial bioagent identifying amplicons include anthranilate synthase component I (trpE), adenylate kinase (adk), adenine glycosylase (mutY), fumarate hydratase (fumC), and pyrophosphate phospho-hydratase (ppa). These 21 primer pairs are indicated with reference to sequence listings in Table 14. Primer pair numbers 1151-1154 hybridize to and amplify segments of trpE. Primer pair numbers 1155-1157 hybridize to and amplify segments of adk. Primer pair numbers 1158-1164 hybridize to and amplify segments of mutY. Primer pair numbers 1165-1170 hybridize to and amplify segments of fumC. Primer pair number 1171 hybridizes to and amplifies a segment of ppa.

Primer pair numbers: 2846-2848 hybridize to and amplify segments of the *parC* gene of DNA topoisomerase which include a codon known to confer quinolone drug resistance upon sub-types of *Acinetobacter baumannii*. Primer pair numbers 2852-2854 hybridize to and amplify segments of the *gyrA* gene of DNA gyrase which include a codon known to confer quinolone drug resistance upon sub-types of *Acinetobacter baumannii*. Primer pair numbers 2922 and 2972 are speciating primers which are useful for identifying different species members of the genus *Acinetobacter*. The primer names given in Table 14A (with the exception of primer pair numbers 2846-2848, 2852-2854) indicate the coordinates to which the primers hybridize to a reference sequence which comprises a concatenation of the genes *TrpE*, *efp* (elongation factor p), *adk*, *mutT*, *fumC*, and *ppa*. For example, the forward primer of primer pair 1151 is named AB_MLST-11-OIF007_62_91_F because it hybridizes to the *Acinetobacter* primer reference sequence of strain type 11 in sample 007 of Operation Iraqi Freedom (OIF) at positions 62 to 91. DNA was sequenced from strain type 11 and from this sequence data and an artificial concatenated sequence of partial gene extractions was assembled for use in design of the triangulation genotyping analysis primers. The stretches of arbitrary residues "N"s in the concatenated sequence were added for the convenience of separation of the partial gene extractions (40N for AB_MLST (SEQ ID NO: 1444)).

[418] The hybridization coordinates of primer pair numbers 2846-2848 are with respect to GenBank Accession number X95819. The hybridization coordinates of primer pair numbers 2852-2854 are with respect to GenBank Accession number AY642140. Sequence residue "T" appearing in the forward and reverse primers of primer pair number 2972 represents inosine.

Table 14A: Triangulation Genotyping Analysis Primer Pairs for Identification of Sub-species characteristics (Strain Type) of Members of the Bacterial Genus *Acinetobacter*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)
1151	AB_MLST-11-OIF007_62_91_F	454	AB_MLST-11-OIF007_169_203_R	1418
1152	AB_MLST-11-OIF007_185_214_F	243	AB_MLST-11-OIF007_291_324_R	959
1153	AB_MLST-11-OIF007_260_289_F	541	AB_MLST-11-OIF007_364_393_R	1400
1154	AB_MLST-11-OIF007_206_239_F	436	AB_MLST-11-OIF007_318_344_R	1016
1155	AB_MLST-11-OIF007_522_552_F	378	AB_MLST-11-OIF007_587_610_R	1392
1156	AB_MLST-11-OIF007_547_571_F	250	AB_MLST-11-OIF007_656_686_R	902
1157	AB_MLST-11-OIF007_601_627_F	256	AB_MLST-11-OIF007_710_736_R	881
1158	AB_MLST-11-OIF007_1202_1225_F	384	AB_MLST-11-OIF007_1266_1296_R	878
1159	AB_MLST-11-OIF007_1202_1225_F	384	AB_MLST-11-OIF007_1299_1316_R	1199
1160	AB_MLST-11-OIF007_1234_1264_F	694	AB_MLST-11-OIF007_1335_1362_R	1215

1161	AB MLST-11-OIF007 1327 1356 F	225	AB MLST-11-OIF007 1422 1448 R	1212
1162	AB MLST-11-OIF007 1345 1369 F	383	AB MLST-11-OIF007 1470 1494 R	1083
1163	AB MLST-11-OIF007 1351 1375 F	662	AB MLST-11-OIF007 1470 1494 R	1083
1164	AB MLST-11-OIF007 1387 1412 F	422	AB MLST-11-OIF007 1470 1494 R	1083
1165	AB MLST-11-OIF007 1542 1569 F	194	AB MLST-11-OIF007 1656 1680 R	1173
1166	AB MLST-11-OIF007 1566 1593 F	684	AB MLST-11-OIF007 1656 1680 R	1173
1167	AB MLST-11-OIF007 1611 1638 F	375	AB MLST-11-OIF007 1731 1757 R	890
1168	AB MLST-11-OIF007 1726 1752 F	182	AB MLST-11-OIF007 1790 1821 R	1195
1169	AB MLST-11-OIF007 1792 1826 F	656	AB MLST-11-OIF007 1876 1909 R	1151
1170	AB MLST-11-OIF007 1792 1826 F	656	AB MLST-11-OIF007 1895 1927 R	1224
1171	AB MLST-11-OIF007 1970 2002 F	618	AB MLST-11-OIF007 2097 2118 R	1157
2846	PARC X95819 33 58 F	302	PARC X95819 121 153 R	852
2847	PARC X95819 33 58 F	199	PARC X95819 157 178 R	889
2848	PARC X95819 33 58 F	596	PARC X95819 97 128 R	1169
2852	GYRA AY642140 -1 24 F	150	GYRA AY642140 71 100 R	1242
2853	GYRA AY642140 25 54 F	166	GYRA AY642140 121 146 R	1069
2854	GYRA AY642140 26 54 F	166	GYRA AY642140 58 89 R	1168
2922	AB MLST-11-OIF007 991 1018 F	583	AB MLST-11-OIF007 1110 1137 R	923
2972	AB MLST-11-OIF007 1007 1034 F	592	AB MLST-11-OIF007 1126 1153 R	924

Table 14B: Triangulation Genotyping Analysis Primer Pairs for Identification of Sub-species characteristics (Strain Type) of Members of the Bacterial Genus *Acinetobacter*

Primer Pair No.	Forward Primer (SEQ ID NO:)	SEQUENCE	Reverse Primer (SEQ ID NO:)	SEQUENCE
1151	454	TGAGATTCTGAACATTTAATGCTGATTGA	1418	TTGTACATTTGAACAATATGCATGACATGTGAAT
1152	243	TATGTGTTCAAACTGTACAAGGTGAAGTGG	969	TCAACAGGTTCTACATCATCAATTTCCATTGCG
1153	541	TGCAGCTTATCAGGTGCCCGAATAATTCG	1400	TTGCATTCGACATATCATTTCCACATGCC
1154	436	TGAAGTGGCTGATGATATCATGCACTTGATGTA	1036	TCCGCCAAAACCTCCCTTTTCCACAG
1155	378	TGCGTTTAGTAAAGAACGATATTGCTCAACC	1392	TTCTGCTTGAGGATAATGTGCTGCG
1156	250	TCAACTGATGTCGCTGAAATGGTGT	903	TACGTTCTACGATTTCTCATCAAGTACATC
1157	256	TCAACGAGAGCTTTGGAAAGAGAGG	881	TACAACTGATAAACAGGACAGAAAGC
1158	384	TGCTGCCCAATTTGCATAAAGC	878	TAAAGCCGGGTAGTGAATCATTTCTTAG
1159	384	TGCTGCCCAATTTGCATAAAGC	1199	TGCACCTGCGTTCAGCG
1160	694	TTTATGACAGCAAGGCAATTTCTGAAAC	1215	TGCCATCATCAATCAAGCCATATGACG
1161	225	TAGGTTTAACTCAGTATGGCGTATATGG	1212	TGCCAGTTTCCATATTCACGTTCTGG
1162	383	TGCTGATATGGAATGCAACGTGAA	1083	TGCCTTGAGTGTATGATGATGCG

1163	662	TTATGAGTGGCAAGTGAAGACCGT	1083	TGCGTTGAGTGTATCATGATTTGCG
1164	422	TCITTGCCATTAAGATGACTTAAGC	1083	TGCGTTGAGTGTATCATGATTTGCG
1165	194	TACTAGCGGTAACTTAACAAGATTGC	1173	TGAGTCGCGTTCACTTTAAGTGGCA
1166	684	TTGCGATGATATTGCTTGGTATGCAAG	1173	TGAGTCGCGTTCACTTTAAGTGGCA
1167	375	TGGCGAATTCGTAATCCGAAATGA	890	TACCGAAGCACGAGCACTTAATAG
1158	182	TACCACTTAATATGTCGCTGCTTC	1195	TGCACTGAATAGATTGAGTAAGTATAAGC
1169	656	TTATACTTACTGCAATCTATTCAGTTGCTGCTG	1151	TGATTTATGCAAGATGATCAATTTCTCAGCA
1170	656	TTATACTTACTGCAATCTATTCAGTTGCTGCTG	1224	TGCGTTAATCAATAGAGGAATTAAGCAAGA
1171	618	TGGTTATGTACCAATACCTTGTCTGAAGATGG	1157	TGAGCGCATGATACCAAGCTC
2846	302	TCCAAAAAATCAGCGTACAGTGG	852	TAAAGCATAGCGGTAACTAAATGCGTACCAT
2847	199	TACTTGGTAAATACCAACCAATGCTGA	883	TACCCGATTTCCCTTGACCTTC
2848	596	TGGTAAATACCAACCAATGCTGAC	1169	TGAGCCATGATACCATGCTCTCATACATGC
2852	190	TAAATCTGCGGTGTGTTGGTGAC	1242	TGCTAAAGTCTTGAGCCATACGAAATAG
2853	166	TAAATCGTAAATATCACCCGATGCTGAC	1069	TGCTATGACGAAATATACCTGACC
2854	166	TAAATCGTAAATATCACCCGATGCTGAC	1168	TGAGCCATACGAAATATGCTTATTAACAGC
2922	583	TGGCGATGCTGCGAATATGTTAAAGA	923	TAGTATCACCAAGTACACCCGATCAGT
2972	592	TGGCGATGCTGCGAATATGTTAAAGA	924	TAGTATCACCAAGTACACCCGATCAGT

[419] Analysis of bioagent identifying amplicons obtained using the primers of Table 14B for over 200 samples from Operation Iraqi Freedom resulted in the identification of 50 distinct strain type clusters. The largest cluster, designated strain type 11 (ST11) includes 42 sample isolates, all of which were obtained from US service personnel and Iraqi civilians treated at the 28th Combat Support Hospital in Baghdad. Several of these individuals were also treated on the hospital ship USNS Comfort. These observations are indicative of significant epidemiological correlation/linkage.

[420] All of the sample isolates were tested against a broad panel of antibiotics to characterize their antibiotic resistance profiles. As an example of a representative result from antibiotic susceptibility testing, ST11 was found to consist of four different clusters of isolates, each with a varying degree of sensitivity/resistance to the various antibiotics tested which included penicillins, extended spectrum penicillins, cephalosporins, carbapenem, protein synthesis inhibitors, nucleic acid synthesis inhibitors, anti-metabolites, and anti-cell membrane antibiotics. Thus, the genotyping power of bacterial bioagent identifying amplicons, particularly drill-down bacterial bioagent identifying amplicons, has the potential to increase the understanding of the transmission of infections in combat casualties, to identify the source of infection in the environment, to track hospital transmission of nosocomial infections, and to

rapidly characterize drug-resistance profiles which enable development of effective infection control measures on a time-scale previously not achievable.

Example 13: Triangulation Genotyping Analysis and Codon Analysis of *Acinetobacter baumannii* Samples from Two Health Care Facilities

[421] In this investigation, 88 clinical samples were obtained from Walter Reed Hospital and 95 clinical samples were obtained from Northwestern Medical Center. All samples from both healthcare facilities were suspected of containing sub-types of *Acinetobacter baumannii*, at least some of which were expected to be resistant to quinolone drugs. Each of the 183 samples was analyzed by the method of the present invention. DNA was extracted from each of the samples and amplified with eight triangulation genotyping analysis primer pairs represented by primer pair numbers: 1151, 1156, 1158, 1160, 1165, 1167, 1170, and 1171. The DNA was also amplified with speciating primer pair number 2922 and codon analysis primer pair numbers 2846-2848 which interrogate a codon present in the *parC* gene, and primer pair numbers 2852-2854 which bracket a codon present in the *gyrA* gene. The *parC* and *gyrA* codon mutations are both responsible for causing drug resistance in *Acinetobacter baumannii*. During evolution of drug resistant strains, the *gyrA* mutation usually occurs before the *parC* mutation. Amplification products were measured by ESI-TOF mass spectrometry as indicated in Example 4. The base compositions of the amplification products were calculated from the average molecular masses of the amplification products and are shown in Tables 15-18. The entries in each of the tables are grouped according to strain type number, which is an arbitrary number assigned to *Acinetobacter baumannii* strains in the order of observance beginning from the triangulation genotyping analysis OIF genotyping study described in Example 12. For example, strain type 11 which appears in samples from the Walter Reed Hospital is the same strain as the strain type 11 mentioned in Example 12. Ibis# refers to the order in which each sample was analyzed. Isolate refers to the original sample isolate numbering system used at the location from which the samples were obtained (either Walter Reed Hospital or Northwestern Medical Center). ST = strain type. ND = not detected. Base compositions highlighted with **bold** type indicate that the base composition is a unique base composition for the amplification product obtained with the pair of primers indicated.

Table 15A: Base Compositions of Amplification Products of 88 *A. baumannii* Samples Obtained from Walter Reed Hospital and Amplified with Codon Analysis Primer Pairs Targeting the *gyrA*

Gene

Species	Ibis#	Isolate	ST	PP No: 2852 <i>gyrA</i>	PP No: 2853 <i>gyrA</i>	PP No: 2854 <i>gyrA</i>
<i>A. baumannii</i>	20	1082	1	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. baumannii</i>	13	854	10	A25G23C21T32	A29G28C21T43	A17G13C13T21

A. baumannii	22	1162	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	27	1230	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	31	1367	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	37	1459	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	55	1700	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	64	1777	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	73	1861	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	74	1877	10	ND	A29G28C21T43	A17G13C13T21
A. baumannii	86	1972	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	3	684	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	6	720	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	7	726	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	19	1079	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	21	1123	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	23	1188	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	33	1417	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	34	1431	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	38	1496	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	40	1523	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	42	1640	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	50	1666	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	51	1668	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	52	1695	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	65	1781	11	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	44	1649	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	49A	1658.1	12	A25G23C22T31	A29G28C21T43	A17G13C13T21
A. baumannii	49B	1658.2	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	56	1707	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	80	1893	12	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	5	693	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	8	749	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	10	839	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	14	865	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	16	888	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	29	1326	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	35	1440	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	41	1524	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	46	1652	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	47	1653	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	48	1657	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	57	1709	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	61	1727	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	63	1762	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	67	1806	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	75	1881	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	77	1886	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	1	649	46	A25G23C21T32	A29G28C21T43	A17G13C13T21

<i>A. baumannii</i>	2	653	46	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	39	1497	16	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	24	1198	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	28	1243	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	43	1648	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	62	1746	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	4	689	15	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	68	1822	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	69	1823A	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	70	1823B	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	71	1826	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	72	1860	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	81	1924	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	82	1929	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	85	1966	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	11	841	3	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. baumannii</i>	32	1415	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	45	1651	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	54	1697	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	58	1712	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	60	1725	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	66	1802	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	76	1883	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	78	1891	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	79	1892	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	83	1947	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	84	1964	24	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	53	1696	24	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. baumannii</i>	36	1458	49	A25G23C21T32	A29G28C21T43	A17G13C13T21
<i>A. baumannii</i>	59	1716	9	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. baumannii</i>	9	805	30	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. baumannii</i>	18	967	39	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. baumannii</i>	30	1322	48	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. baumannii</i>	26	1218	50	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. sp. 13TU</i>	15	875	A1	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. sp. 13TU</i>	17	895	A1	A25G23C22T31	A29G28C22T42	A17G13C14T20
<i>A. sp. 3</i>	12	853	B7	A25G22C22T32	A30G29C22T40	A17G13C14T20
<i>A. johnsonii</i>	25	1202	NEW1	A25G22C22T32	A30G29C22T40	A17G13C14T20
<i>A. sp. 2082</i>	87	2082	NEW2	A25G22C22T32	A31G28C22T40	A17G13C14T20

Table 15B: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Codon Analysis Primer Pairs Targeting the parC Gene

Species	Ibis#	Isolate	ST	PP No: 2846 parC	PP No: 2847 parC	PP No: 2848 parC
<i>A. baumannii</i>	20	1082	1	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	13	854	10	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	22	1162	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	27	1230	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	31	1367	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	37	1459	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	55	1700	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	64	1777	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	73	1861	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	74	1877	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	86	1972	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	3	684	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	6	720	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	7	726	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	19	1079	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	21	1123	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	23	1188	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	33	1417	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	34	1431	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	38	1496	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	40	1523	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	42	1640	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	50	1666	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	51	1668	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	52	1695	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	65	1781	11	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	44	1649	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	49A	1658.1	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	49B	1658.2	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	56	1707	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	80	1893	12	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	5	693	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	8	749	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	10	839	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	14	865	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	16	888	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	29	1326	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	35	1440	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	41	1524	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	46	1652	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	47	1653	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	48	1657	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	57	1709	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	61	1727	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	63	1762	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	67	1806	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	75	1881	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	77	1886	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	1	649	46	A33G26C28T34	A29G28C25T32	A16G14C14T16

<i>A. baumannii</i>	2	653	46	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	39	1497	16	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	24	1198	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
<i>A. baumannii</i>	28	1243	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
<i>A. baumannii</i>	43	1648	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
<i>A. baumannii</i>	62	1746	15	A33G26C28T34	A29G29C23T33	A16G14C14T16
<i>A. baumannii</i>	4	689	15	A34G25C29T33	A30G27C26T31	A16G14C15T15
<i>A. baumannii</i>	68	1822	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	69	1823A	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	70	1823B	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	71	1826	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	72	1860	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	81	1924	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	82	1929	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	85	1966	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	11	841	3	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	32	1415	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	45	1651	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	54	1697	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	58	1712	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	60	1725	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	66	1802	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	76	1883	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	78	1891	24	A34G25C29T33	A30G27C26T31	A16G14C15T15
<i>A. baumannii</i>	79	1892	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	83	1947	24	A34G25C29T33	A30G27C26T31	A16G14C15T15
<i>A. baumannii</i>	84	1964	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	53	1696	24	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	36	1458	49	A34G26C29T32	A30G28C24T32	A16G14C15T15
<i>A. baumannii</i>	59	1716	9	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	9	805	30	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	18	967	39	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	30	1322	48	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	26	1218	50	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. sp. 137U</i>	15	875	A1	A32G26C28T35	A28G28C24T34	A16G14C15T15
<i>A. sp. 137U</i>	17	895	A1	A32G26C28T35	A28G28C24T34	A16G14C15T15
<i>A. sp. 3</i>	12	853	B7	A29G26C27T39	A26G32C21T35	A16G14C15T15
<i>A. johnsonii</i>	25	1202	NEW1	A32G28C26T35	A29G29C22T34	A16G14C15T15
<i>A. sp. 2082</i>	87	2082	NEW2	A33G27C26T35	A31G28C20T35	A16G14C15T15

Table 16A: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Codon Analysis Primer Pairs Targeting the *gyrA* Gene

Species	Ibis#	Isolate	ST	PP No: 2852 <i>gyrA</i>	PP No: 2853 <i>gyrA</i>	PP No: 2854 <i>gyrA</i>
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A. baumannii	54	536	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	87	665	3	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	8	80	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	9	91	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	10	92	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	11	131	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	12	137	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	21	218	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	26	242	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	94	678	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	1	9	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	2	13	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	3	19	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	4	24	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	5	36	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	6	39	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	13	139	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	15	165	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	16	170	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	17	186	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	20	202	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	22	221	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	24	234	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	25	239	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	33	370	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	34	389	10	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	19	201	14	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	27	257	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	29	301	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	31	354	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	36	422	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	37	424	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	38	434	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	39	473	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	40	482	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	44	512	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	45	516	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	47	522	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	48	526	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	50	528	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	52	531	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	53	533	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	56	542	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	59	550	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	62	556	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	64	557	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	70	588	51	A25G23C21T32	A29G28C21T43	A17G13C13T21

A. baumannii	73	603	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	74	605	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	75	606	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	77	611	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	79	622	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	83	643	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	85	653	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	89	669	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	93	674	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	23	228	51	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	32	369	52	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	35	393	52	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	30	339	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	41	485	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	42	493	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	43	502	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	46	520	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	49	527	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	51	529	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	65	562	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	68	579	53	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	57	546	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	58	548	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	60	552	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	61	555	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	63	557	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	66	570	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	67	578	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	69	584	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	71	593	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	72	602	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	76	609	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	78	621	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	80	625	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	81	628	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	82	632	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	84	649	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	86	655	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	88	668	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	90	671	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	91	672	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	92	673	54	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	18	196	55	A25G23C22T31	A29G28C21T43	A17G13C13T21
A. baumannii	55	537	27	A25G23C21T32	A29G28C21T43	A17G13C13T21
A. baumannii	28	263	27	A25G23C22T31	A29G28C22T42	A17G13C14T20
A. sp. 3	14	164	B7	A25G22C22T32	A30G29C22T40	A17G13C14T20
mixture	7	71	-	ND	ND	A17G13C15T19

Table 16B: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Codon Analysis Primer Pairs Targeting the *parC* Gene

Species	Ibis#	Isolate	ST	PP No: 2846 parC	PP No: 2847 parC	PP No: 2848 parC
<i>A. baumannii</i>	54	536	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	87	665	3	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	8	80	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	9	91	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	10	92	10	A33G26C28T34	A29G28C25T32	ND
<i>A. baumannii</i>	11	131	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	12	137	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	21	218	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	26	242	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	94	678	10	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	1	9	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	2	13	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	3	19	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	4	24	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	5	36	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	6	39	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	13	139	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	15	165	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	16	170	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	17	186	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	20	202	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	22	221	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	24	234	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	25	239	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	33	370	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	34	389	10	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	19	201	14	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	27	257	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	29	301	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	31	354	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	36	422	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	37	424	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	38	434	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	39	473	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	40	482	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	44	512	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	45	516	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	47	522	51	A33G26C28T34	A29G28C25T32	A16G14C14T16

A. baumannii	48	526	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	50	528	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	52	531	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	53	533	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	56	542	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	59	550	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	62	556	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	64	557	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	70	588	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	73	603	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	74	605	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	75	606	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	77	611	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	79	622	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	83	643	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	85	653	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	89	669	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	93	674	51	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	23	228	51	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	32	369	52	A34G25C28T34	A30G27C25T32	A16G14C14T16
A. baumannii	35	393	52	A34G25C28T34	A30G27C25T32	A16G14C14T16
A. baumannii	30	339	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	41	485	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	42	493	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	43	502	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	46	520	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	49	527	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	51	529	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	65	562	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	68	579	53	A34G25C29T33	A30G27C26T31	A16G14C15T15
A. baumannii	57	546	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	58	548	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	60	552	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	61	555	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	63	557	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	66	570	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	67	578	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	69	584	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	71	593	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	72	602	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	76	609	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	78	621	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	80	625	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	81	628	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	82	632	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	84	649	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
A. baumannii	86	655	54	A33G26C28T34	A29G28C25T32	A16G14C14T16

<i>A. baumannii</i>	88	668	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	90	671	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	91	672	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	92	673	54	A33G26C28T34	A29G28C25T32	A16G14C14T16
<i>A. baumannii</i>	18	196	55	A33G27C28T33	A29G28C25T31	A15G14C15T16
<i>A. baumannii</i>	55	537	27	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. baumannii</i>	28	263	27	A33G26C29T33	A29G28C26T31	A16G14C15T15
<i>A. sp. 3</i>	14	164	B7	A35G25C29T32	A30G28C17T39	A16G14C15T15
mixture	7	71	-	ND	ND	A17G14C15T14

Table 17A: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Speciating Primer Pair No. 2922 and Triangulation Genotyping Analysis Primer Pair Nos. 1151 and 1156

Species	Ibis#	Isolate	ST	PP No: 2922 efp	PP No: 1151 trpE	PP No: 1156 Adk
<i>A. baumannii</i>	20	1082	1	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	13	854	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	22	1162	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	27	1230	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	31	1367	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	37	1459	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	55	1700	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	64	1777	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	73	1861	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	74	1877	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	86	1972	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	3	684	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	6	720	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	7	726	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	19	1079	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	21	1123	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	23	1188	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	33	1417	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	34	1431	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	38	1496	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	40	1523	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	42	1640	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	50	1666	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	51	1668	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	52	1695	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	65	1781	11	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	44	1649	12	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	49A	1658.1	12	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	49B	1658.2	12	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	56	1707	12	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	80	1893	12	A45G34C25T43	A44G35C21T42	A44G32C26T38

A. baumannii	5	693	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	8	749	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	10	839	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	14	865	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	16	888	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	29	1326	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	35	1440	14	A44G35C25T43	ND	A44G32C27T37
A. baumannii	41	1524	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	46	1652	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	47	1653	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	48	1657	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	57	1709	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	61	1727	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	63	1762	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	67	1806	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	75	1881	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	77	1886	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	1	649	46	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	2	653	46	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	39	1497	16	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	24	1198	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	28	1243	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	43	1648	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	62	1746	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	4	689	15	A44G35C25T43	A44G35C22T41	A44G32C26T38
A. baumannii	68	1822	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	69	1823A	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	70	1823B	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	71	1826	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	72	1860	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	81	1924	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	82	1929	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	85	1966	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	11	841	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
A. baumannii	32	1415	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	45	1651	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	54	1697	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	58	1712	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	60	1725	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	66	1802	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	76	1883	24	ND	A43G36C20T43	A44G32C27T37
A. baumannii	78	1891	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	79	1892	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	83	1947	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	84	1964	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	53	1696	24	A44G35C25T43	A43G36C20T43	A44G32C27T37
A. baumannii	36	1458	49	A44G35C25T43	A44G35C22T41	A44G32C27T37

<i>A. baumannii</i>	59	1716	9	A44G35C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	9	805	30	A44G35C25T43	A44G35C19T44	A44G32C27T37
<i>A. baumannii</i>	18	967	39	A45G34C25T43	A44G35C22T41	A44G32C26T38
<i>A. baumannii</i>	30	1322	48	A44G35C25T43	A43G36C20T43	A44G32C27T37
<i>A. baumannii</i>	26	1218	50	A44G35C25T43	A44G35C21T42	A44G32C26T38
<i>A. sp. 13TU</i>	15	875	A1	A47G33C24T43	A46G32C20T44	A44G33C27T36
<i>A. sp. 13TU</i>	17	895	A1	A47G33C24T43	A46G32C20T44	A44G33C27T36
<i>A. sp. 3</i>	12	853	B7	A46G35C24T42	A42G34C20T46	A43G33C24T40
<i>A. johnsonii</i>	25	1202	NEW1	A46G35C23T43	A42G35C21T44	A43G33C23T41
<i>A. sp. 2082</i>	87	2082	NEW2	A46G36C22T43	A42G32C20T48	A42G34C23T41

Table 17B: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Triangulation Genotyping Analysis Primer Pair Nos.

1158 and 1160 and 1165

Species	Ibis#	Isolate	ST	PP No: 1158 mutY	PP No: 1160 mutY	PP No: 1165 fumC
<i>A. baumannii</i>	20	1082	1	A27G21C25T22	A32G35C29T33	A40G33C30T36
<i>A. baumannii</i>	13	854	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	22	1162	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	27	1230	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	31	1367	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	37	1459	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	55	1700	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	64	1777	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	73	1861	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	74	1877	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	86	1972	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	3	684	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	6	720	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	7	726	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	19	1079	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	21	1123	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	23	1188	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	33	1417	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	34	1431	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	38	1496	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	40	1523	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	42	1640	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	50	1666	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	51	1668	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	52	1695	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	65	1781	11	A27G21C25T22	A32G34C28T35	A40G33C30T36
<i>A. baumannii</i>	44	1649	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
<i>A. baumannii</i>	49A	1658.1	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
<i>A. baumannii</i>	49B	1658.2	12	A27G21C26T21	A32G34C29T34	A40G33C30T36

A. baumannii	56	1707	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	80	1893	12	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	5	693	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	8	749	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	10	839	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	14	865	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	16	888	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	29	1326	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	35	1440	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	41	1524	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	46	1652	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	47	1653	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	48	1657	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	57	1709	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	61	1727	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	63	1762	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	67	1806	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	75	1881	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	77	1886	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	1	649	46	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	2	653	46	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	39	1497	16	A29G19C26T21	A31G35C29T34	A40G34C29T36
A. baumannii	24	1198	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	28	1243	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	43	1648	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	62	1746	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	4	689	15	A29G19C26T21	A31G35C29T34	A40G33C29T37
A. baumannii	68	1822	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	69	1823A	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	70	1823B	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	71	1826	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	72	1860	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	81	1924	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	82	1929	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	85	1966	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	11	841	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
A. baumannii	32	1415	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	45	1651	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	54	1697	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	58	1712	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	60	1725	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	66	1802	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	76	1883	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	78	1891	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	79	1892	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	83	1947	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	84	1964	24	A27G21C26T21	A32G35C28T34	A40G33C30T36

<i>A. baumannii</i>	53	1696	24	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	36	1458	49	A27G20C27T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	59	1716	9	A27G21C25T22	A32G35C28T34	A39G33C30T37
<i>A. baumannii</i>	9	805	30	A27G21C25T22	A32G35C28T34	A39G33C30T37
<i>A. baumannii</i>	18	967	39	A27G21C26T21	A32G35C28T34	A39G33C30T37
<i>A. baumannii</i>	30	1322	48	A28G21C24T22	A32G35C29T33	A40G33C30T36
<i>A. baumannii</i>	26	1218	50	A27G21C25T22	A31G36C28T34	A40G33C29T37
<i>A. sp. 13TU</i>	15	875	A1	A27G21C25T22	A30G36C26T37	A41G34C28T36
<i>A. sp. 13TU</i>	17	895	A1	A27G21C25T22	A30G36C26T37	A41G34C28T36
<i>A. sp. 3</i>	12	853	B7	A26G23C23T23	A30G36C27T36	A39G37C26T37
<i>A. johnsonii</i>	25	1202	NEW1	A25G23C24T23	A30G35C30T34	A38G37C26T38
<i>A. sp. 2082</i>	87	2082	NEW2	A26G22C24T23	A31G35C28T35	A42G34C27T36

Table 17C: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Walter Reed Hospital and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1167 and 1170 and 1171

Species	Ibis#	Isolate	ST	PP No: 1167 fumC	PP No: 1170 fumC	PP No: 1171 ppa
<i>A. baumannii</i>	20	1082	1	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	13	854	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	22	1162	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	27	1230	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	31	1367	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	37	1459	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	55	1700	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	64	1777	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	73	1861	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	74	1877	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	86	1972	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	3	684	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	6	720	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	7	726	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	19	1079	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	21	1123	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	23	1188	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	33	1417	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	34	1431	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	38	1496	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	40	1523	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	42	1640	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	50	1666	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	51	1668	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	52	1695	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	65	1781	11	A41G34C34T38	A38G27C21T50	A35G37C33T44
<i>A. baumannii</i>	44	1649	12	A41G34C34T38	A38G27C21T50	A35G37C33T44

A. baumannii	49A	1658.1	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	49B	1658.2	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	56	1707	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	80	1893	12	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	5	693	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	8	749	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	10	839	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	14	865	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	16	888	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	29	1326	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	35	1440	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	41	1524	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	46	1652	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	47	1653	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	48	1657	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	57	1709	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	61	1727	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	63	1762	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	67	1806	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	75	1881	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	77	1886	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	1	649	46	A41G35C32T39	A37G28C20T51	A35G37C32T45
A. baumannii	2	653	46	A41G35C32T39	A37G28C20T51	A35G37C32T45
A. baumannii	39	1497	16	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	24	1198	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	28	1243	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	43	1648	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	62	1746	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	4	689	15	A41G35C32T39	A37G28C20T51	A35G37C30T47
A. baumannii	68	1822	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	69	1823A	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	70	1823B	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	71	1826	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	72	1860	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	81	1924	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	82	1929	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	85	1966	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	11	841	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	32	1415	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	45	1651	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	54	1697	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	58	1712	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	60	1725	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	66	1802	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	76	1883	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	78	1891	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
A. baumannii	79	1892	24	A40G35C34T38	A39G26C22T49	A35G37C33T44

<i>A. baumannii</i>	83	1947	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
<i>A. baumannii</i>	84	1964	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
<i>A. baumannii</i>	53	1696	24	A40G35C34T38	A39G26C22T49	A35G37C33T44
<i>A. baumannii</i>	36	1458	49	A40G35C34T38	A39G26C22T49	A35G37C30T47
<i>A. baumannii</i>	59	1716	9	A40G35C32T40	A38G27C20T51	A36G35C31T47
<i>A. baumannii</i>	9	805	30	A40G35C32T40	A38G27C21T50	A35G36C29T49
<i>A. baumannii</i>	18	967	39	A40G35C33T39	A38G27C20T51	A35G37C30T47
<i>A. baumannii</i>	30	1322	48	A40G35C35T37	A38G27C21T50	A35G37C30T47
<i>A. baumannii</i>	26	1218	50	A40G35C34T38	A38G27C21T50	A35G37C33T44
<i>A. sp. 13TU</i>	15	875	A1	A41G39C31T36	A37G26C24T49	A34G38C31T46
<i>A. sp. 13TU</i>	17	895	A1	A41G39C31T36	A37G26C24T49	A34G38C31T46
<i>A. sp. 3</i>	12	853	B7	A43G37C30T37	A36G27C24T49	A34G37C31T47
<i>A. johnsonii</i>	25	1202	NEW1	A42G38C31T36	A40G27C19T50	A35G37C32T45
<i>A. sp. 2082</i>	87	2082	NEW2	A43G37C32T35	A37G26C21T52	A35G38C31T45

Table 18A: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Speciating Primer Pair No. 2922 and Triangulation Genotyping Analysis Primer Pair Nos. 1151 and 1156

Species	Ibis#	Isolate	ST	PP No: 2922 efp	PP No: 1151 trpE	PP No: 1156 adk
<i>A. baumannii</i>	54	536	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
<i>A. baumannii</i>	87	665	3	A44G35C24T44	A44G35C22T41	A44G32C26T38
<i>A. baumannii</i>	8	80	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	9	91	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	10	92	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	11	131	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	12	137	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	21	218	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	26	242	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	94	678	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	1	9	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	2	13	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	3	19	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	4	24	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	5	36	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	6	39	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	13	139	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	15	165	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	16	170	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	17	186	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	20	202	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	22	221	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	24	234	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	25	239	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
<i>A. baumannii</i>	33	370	10	A45G34C25T43	A44G35C21T42	A44G32C26T38

A. baumannii	34	389	10	A45G34C25T43	A44G35C21T42	A44G32C26T38
A. baumannii	19	201	14	A44G35C25T43	A44G35C22T41	A44G32C27T37
A. baumannii	27	257	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	29	301	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	31	354	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	36	422	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	37	424	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	38	434	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	39	473	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	40	482	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	44	512	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	45	516	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	47	522	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	48	526	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	50	528	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	52	531	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	53	533	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	56	542	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	59	550	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	62	556	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	64	557	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	70	588	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	73	603	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	74	605	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	75	606	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	77	611	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	79	622	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	83	643	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	85	653	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	89	669	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	93	674	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	23	228	51	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	32	369	52	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	35	393	52	A44G35C25T43	A43G36C20T43	A44G32C26T38
A. baumannii	30	339	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	41	485	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	42	493	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	43	502	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	46	520	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	49	527	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	51	529	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	65	562	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	68	579	53	A44G35C25T43	A44G35C19T44	A44G32C27T37
A. baumannii	57	546	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	58	548	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	60	552	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
A. baumannii	61	555	54	A44G35C25T43	A44G35C20T43	A44G32C26T38

<i>A. baumannii</i>	63	557	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	66	570	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	67	578	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	69	584	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	71	593	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	72	602	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	76	609	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	78	621	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	80	625	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	81	628	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	82	632	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	84	649	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	86	655	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	88	668	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	90	671	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	91	672	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	92	673	54	A44G35C25T43	A44G35C20T43	A44G32C26T38
<i>A. baumannii</i>	18	196	55	A44G35C25T43	A44G35C20T43	A44G32C27T37
<i>A. baumannii</i>	55	537	27	A44G35C25T43	A44G35C19T44	A44G32C27T37
<i>A. baumannii</i>	28	263	27	A44G35C25T43	A44G35C19T44	A44G32C27T37
<i>A. sp. 3</i>	14	164	B7	A46G35C24T42	A42G34C20T46	A43G33C24T40
mixture	7	71	?	mixture	ND	ND

Table 18B: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1158, 1160 and 1165

Species	Ibis#	Isolate	ST	PP No: 1158 mutY	PP No: 1160 mutY	PP No: 1165 fumC
<i>A. baumannii</i>	54	536	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	87	665	3	A27G20C27T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	8	80	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	9	91	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	10	92	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	11	131	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	12	137	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	21	218	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	26	242	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	94	678	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	1	9	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	2	13	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	3	19	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	4	24	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	5	36	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	6	39	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	13	139	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
<i>A. baumannii</i>	15	165	10	A27G21C26T21	A32G35C28T34	A40G33C30T36

A. baumannii	16	170	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	17	186	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	20	202	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	22	221	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	24	234	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	25	239	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	33	370	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	34	389	10	A27G21C26T21	A32G35C28T34	A40G33C30T36
A. baumannii	19	201	14	A27G21C25T22	A31G36C28T34	A40G33C29T37
A. baumannii	27	257	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	29	301	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	31	354	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	36	422	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	37	424	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	38	434	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	39	473	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	40	482	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	44	512	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	45	516	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	47	522	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	48	526	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	50	528	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	52	531	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	53	533	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	56	542	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	59	550	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	62	556	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	64	557	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	70	588	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	73	603	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	74	605	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	75	606	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	77	611	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	79	622	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	83	643	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	85	653	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	89	669	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	93	674	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	23	228	51	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	32	369	52	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	35	393	52	A27G21C25T22	A32G35C28T34	A40G33C29T37
A. baumannii	30	339	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	41	485	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	42	493	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	43	502	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	46	520	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	49	527	53	A28G20C26T21	A32G34C29T34	A40G33C30T36

A. baumannii	51	529	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	65	562	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	68	579	53	A28G20C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	57	546	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	58	548	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	60	552	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	61	555	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	63	557	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	66	570	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	67	578	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	69	584	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	71	593	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	72	602	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	76	609	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	78	621	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	80	625	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	81	628	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	82	632	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	84	649	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	86	655	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	88	668	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	90	671	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	91	672	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	92	673	54	A27G21C26T21	A32G34C29T34	A40G33C30T36
A. baumannii	18	196	55	A27G21C25T22	A31G36C27T35	A40G33C29T37
A. baumannii	55	537	27	A27G21C25T22	A32G35C28T34	A40G33C30T36
A. baumannii	28	263	27	A27G21C25T22	A32G35C28T34	A40G33C30T36
A. sp. 3	14	164	B7	A26G23C23T23	A30G36C27T36	A39G37C26T37
mixture	7	71	?	ND	ND	ND

Table 18C: Base Compositions Determined from *A. baumannii* DNA Samples Obtained from Northwestern Medical Center and Amplified with Triangulation Genotyping Analysis Primer Pair Nos. 1167, 1170 and 1171

Species	Ibis#	Isolate	ST	PP No: 1167 fumC	PP No: 1170 fumC	PP No: 1171 ppa
A. baumannii	54	536	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	87	665	3	A41G34C35T37	A38G27C20T51	A35G37C31T46
A. baumannii	8	80	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	9	91	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	10	92	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	11	131	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	12	137	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	21	218	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	26	242	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	94	678	10	A41G34C34T38	A38G27C21T50	A35G37C33T44

A. baumannii	1	9	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	2	13	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	3	19	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	4	24	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	5	36	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	6	39	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	13	139	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	15	165	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	16	170	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	17	186	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	20	202	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	22	221	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	24	234	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	25	239	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	33	370	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	34	389	10	A41G34C34T38	A38G27C21T50	A35G37C33T44
A. baumannii	19	201	14	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	27	257	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	29	301	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	31	354	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	36	422	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	37	424	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	38	434	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	39	473	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	40	482	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	44	512	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	45	516	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	47	522	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	48	526	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	50	528	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	52	531	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	53	533	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	56	542	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	59	550	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	62	556	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	64	557	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	70	588	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	73	603	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	74	605	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	75	606	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	77	611	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	79	622	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	83	643	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	85	653	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	89	669	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	93	674	51	A40G35C34T38	A38G27C21T50	A35G37C30T47
A. baumannii	23	228	51	A40G35C34T38	A38G27C21T50	A35G37C30T47

<i>A. baumannii</i>	32	369	52	A40G35C34T38	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	35	393	52	A40G35C34T38	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	30	339	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	41	485	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	42	493	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	43	502	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	46	520	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	49	527	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	51	529	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	65	562	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	68	579	53	A40G35C35T37	A38G27C21T50	A35G37C31T46
<i>A. baumannii</i>	57	546	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	58	548	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	60	552	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	61	555	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	63	557	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	66	570	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	67	578	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	69	584	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	71	593	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	72	602	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	76	609	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	78	621	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	80	625	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	81	628	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	82	632	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	84	649	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	86	655	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	88	668	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	90	671	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	91	672	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	92	673	54	A40G35C34T38	A39G26C22T49	A35G37C31T46
<i>A. baumannii</i>	18	196	55	A42G34C33T38	A38G27C20T51	A35G37C31T46
<i>A. baumannii</i>	55	537	27	A40G35C33T39	A38G27C20T51	A35G37C33T44
<i>A. baumannii</i>	28	263	27	A40G35C33T39	A38G27C20T51	A35G37C33T44
<i>A. sp. 3</i>	14	164	B7	A43G37C30T37	A36G27C24T49	A34G37C31T47
mixture	7	71	-	ND	ND	ND

[422] Base composition analysis of the samples obtained from Walter Reed hospital indicated that a majority of the strain types identified were the same strain types already characterized by the OIF study of Example 12. This is not surprising since at least some patients from which clinical samples were obtained in OIF were transferred to the Walter Reed Hospital (WRAIR). Examples of these common strain types include: ST10, ST11, ST12, ST14, ST15, ST16 and ST46. A strong correlation was noted between these strain types and the presence of mutations in the *gyrA* and *parC* which confer quinolone drug resistance.

[423] In contrast, the results of base composition analysis of samples obtained from Northwestern Medical Center indicate the presence of 4 major strain types: ST10, ST51, ST53 and ST54. All of these strain types have the *gyrA* quinolone resistance mutation and most also have the *parC* quinolone resistance mutation, with the exception of ST35. This observation is consistent with the current understanding that the *gyrA* mutation generally appears before the *parC* mutation and suggests that the acquisition of these drug resistance mutations is rather recent and that resistant isolates are taking over the wild-type isolates. Another interesting observation was that a single isolate of ST3 (isolate 841) displays a triangulation genotyping analysis pattern similar to other isolates of ST3, but the codon analysis amplification product base compositions indicate that this isolate has not yet undergone the quinolone resistance mutations in *gyrA* and *parC*.

[424] The six isolates that represent species other than *Acinetobacter baumannii* in the samples obtained from the Walter Reed Hospital were each found to not carry the drug resistance mutations.

[425] The results described above involved analysis of 183 samples using the methods and compositions of the present invention. Results were provided to collaborators at the Walter Reed hospital and Northwestern Medical center within a week of obtaining samples. This example highlights the rapid throughput characteristics of the analysis platform and the resolving power of triangulation genotyping analysis and codon analysis for identification of and determination of drug resistance in bacteria.

Example 14: Identification of Drug Resistance Genes and Virulence Factors in *Staphylococcus aureus*

[426] An eight primer pair panel was designed for identification of drug resistance genes and virulence factors of *Staphylococcus aureus* and is shown in Table 19. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 19.

Table 19: Primer Pairs for Identification of Drug Resistance Genes and Virulence Factors in *Staphylococcus aureus*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
879	MECA_Y14051_4507_4530 F	288	MECA_Y14051_4555_4581 R	1269	meaA
2056	MECI-R_NC003923-41798-41609_33_60 F	698	MECI-R_NC003923-41798-41609_86_113 R	1420	MecI-R
2081	ERM_A_NC002952-55890-	217	ERM_A_NC002952-55890-	1167	ermA

	56621_366_395_F		56621_438_465_R		
2086	ERM_C0005908-2004- 2738_85_116_F	399	ERM_C0005908-2004- 2738_173_206_R	1041	ermC
2095	PVL_C0003923-1529595- 1531285_688_713_F	456	PVL_C0003923-1529595- 1531285_775_804_R	1261	Pv-luk
2249	TUF_C0002758-615038- 616222_696_725_F	430	TUF_C0002758-615038- 616222_793_820_R	1321	tufB
2256	NUC_C0002758-894288- 894974_316_345_F	174	NUC_C0002758-894288- 894974_396_421_R	853	Nuc
2313	MUP_C00075439_2486_2516_F	172	MUP_C00075439_2548_2574_R	1360	mupR

[427] Primer pair numbers 2256 and 2249 are confirmation primers designed with the aim of high level identification of *Staphylococcus aureus*. The nuc gene is a *Staphylococcus aureus*-specific marker gene. The tufB gene is a universal housekeeping gene but the bioagent identifying amplicon defined by primer pair number 2249 provides a unique base composition (A43 G28 C19 T35) which distinguishes *Staphylococcus aureus* from other members of the genus *Staphylococcus*.

[428] High level methicillin resistance in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair numbers 879 and 2056. Analyses have indicated that primer pair number 879 is not expected to prime *S. sciuri* homolog or *Enterococcus faecalis/facium* ampicillin-resistant PBP5 homologs.

[429] Macrolide and erythromycin resistance in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair numbers 2081 and 2086.

[430] Resistance to mupirocin in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair number 2313.

[431] Virulence in a given strain of *Staphylococcus aureus* is indicated by bioagent identifying amplicons defined by primer pair number 2095. This primer pair can simultaneously identify the pvl (lukS-PV) gene and the lukD gene which encodes a homologous enterotoxin. A bioagent identifying amplicon of the lukD gene has a six nucleobase length difference relative to the lukS-PV gene.

[432] A total of 32 blinded samples of different strains of *Staphylococcus aureus* were provided by the Center for Disease Control (CDC). Each sample was analyzed by PCR amplification with the eight primer pair panel, followed by purification and measurement of molecular masses of the amplification products by mass spectrometry. Base compositions for the amplification products were calculated. The base compositions provided the information summarized above for each primer pair. The results are shown in Tables 20A and B. One result noted upon un-blinding of the samples is that each of the PVL+ identifications agreed with PVL+ identified in the same samples by standard PCR assays. These results

indicate that the panel of eight primer pairs is useful for identification of drug resistance and virulence sub-species characteristics for *Staphylococcus aureus*. It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Table 20A: Drug Resistance and Virulence Identified in Blinded Samples of Various Strains of *Staphylococcus aureus* with Primer Pair Nos. 2081, 2086, 2095 and 2256

Sample Index No.	Primer Pair No. 2081 (exmA)	Primer Pair No. 2086 (exmC)	Primer Pair No. 2095 (pv-luk)	Primer Pair No. 2256 (nuc)
CDC0010	-	-	PVL-/lukD+	+
CDC0015	-	-	PVL+/lukD+	+
CDC0019	-	+	PVL-/lukD+	+
CDC0026	+	-	PVL-/lukD+	+
CDC0030	+	-	PVL-/lukD+	+
CDC004	-	-	PVL+/lukD+	+
CDC0014	-	+	PVL+/lukD+	+
CDC008	-	-	PVL-/lukD+	+
CDC001	+	-	PVL-/lukD+	+
CDC0022	+	-	PVL-/lukD+	+
CDC006	+	-	PVL-/lukD+	+
CDC007	-	-	PVL-/lukD+	+
CDCVRS1	+	-	PVL-/lukD+	+
CDCVRS2	+	+	PVL-/lukD+	+
CDC0011	+	-	PVL-/lukD+	+
CDC0012	-	-	<u>PVL+/lukD-</u>	+
CDC0021	+	-	PVL-/lukD+	+
CDC0023	+	-	PVL-/lukD+	+
CDC0025	+	-	PVL-/lukD+	+
CDC005	-	-	PVL-/lukD+	+
CDC0018	+	-	<u>PVL+/lukD-</u>	+
CDC002	-	-	PVL-/lukD+	+
CDC0028	+	-	PVL-/lukD+	+
CDC003	-	-	PVL-/lukD+	+
CDC0013	-	-	PVL+/lukD+	+
CDC0016	-	-	PVL-/lukD+	+
CDC0027	+	-	PVL-/lukD+	+
CDC0029	-	-	PVL+/lukD+	+

CDC0020	-	+	FVL-/lukD+	+
CDC0024	-	-	FVL-/lukD+	+
CDC0031	-	-	FVL-/lukD+	+

Table 20B: Drug Resistance and Virulence Identified in Blinded Samples of Various Strains of *Staphylococcus aureus* with Primer Pair Nos. 2249, 879, 2056, and 2313

Sample Index No.	Primer Pair No. 2249 (tufB)	Primer Pair No. 879 (mecA)	Primer Pair No. 2056 (mecI-R)	Primer Pair No. 2313 (mupR)
CDC0010	<i>Staphylococcus aureus</i>	+	+	-
CDC0015	<i>Staphylococcus aureus</i>	-	-	-
CDC0019	<i>Staphylococcus aureus</i>	+	+	-
CDC0026	<i>Staphylococcus aureus</i>	+	+	-
CDC0030	<i>Staphylococcus aureus</i>	+	+	-
CDC004	<i>Staphylococcus aureus</i>	+	+	-
CDC0014	<i>Staphylococcus aureus</i>	+	+	-
CDC008	<i>Staphylococcus aureus</i>	+	+	-
CDC001	<i>Staphylococcus aureus</i>	+	+	-
CDC0022	<i>Staphylococcus aureus</i>	+	+	+
CDC006	<i>Staphylococcus aureus</i>	+	+	-
CDC007	<i>Staphylococcus aureus</i>	+	+	-
CDCVRS1	<i>Staphylococcus aureus</i>	+	+	-
CDCVRS2	<i>Staphylococcus aureus</i>	+	+	-
CDC0011	<i>Staphylococcus aureus</i>	-	-	-
CDC0012	<i>Staphylococcus aureus</i>	+	+	-
CDC0021	<i>Staphylococcus aureus</i>	+	+	-
CDC0023	<i>Staphylococcus aureus</i>	+	+	-
CDC0025	<i>Staphylococcus aureus</i>	+	+	-
CDC005	<i>Staphylococcus aureus</i>	+	+	-
CDC0018	<i>Staphylococcus aureus</i>	+	+	-
CDC002	<i>Staphylococcus aureus</i>	+	+	-
CDC0028	<i>Staphylococcus aureus</i>	+	+	-
CDC003	<i>Staphylococcus aureus</i>	+	+	-
CDC0013	<i>Staphylococcus aureus</i>	+	+	-
CDC0016	<i>Staphylococcus aureus</i>	+	+	-
CDC0027	<i>Staphylococcus aureus</i>	+	+	-
CDC0029	<i>Staphylococcus aureus</i>	+	+	-

CDC0020	<i>Staphylococcus aureus</i>	-	-	-
CDC0024	<i>Staphylococcus aureus</i>	+	+	-
CDC0031	<i>Staphylococcus schleiferi</i>	-	-	-

Example 15: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for *Staphylococcus aureus*

[433] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of eight triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within six different housekeeping genes which are listed in Table 21. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 21.

Table 21: Primer Pairs for Triangulation Genotyping Analysis of *Staphylococcus aureus*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
2146	ARCC_NC003923-2725050-2724595 131 161 F	437	ARCC_NC003923-2725050-2724595 214 245 R	1137	arcC
2149	ARO_E_NC003923-1674726-1674277 30 62 F	530	ARO_E_NC003923-1674726-1674277 155 181 R	891	aroE
2150	ARO_E_NC003923-1674726-1674277 204 232 F	474	ARO_E_NC003923-1674726-1674277 308 335 R	869	aroE
2156	GMK_NC003923-1190906-1191334 301 329 F	268	GMK_NC003923-1190906-1191334 403 432 R	1284	gmK
2157	PTA_NC003923-628885-629355 237 263 F	418	PTA_NC003923-628885-629355 314 345 R	1301	pta
2161	TP1_NC003923-830671-831072 1 34 F	318	TP1_NC003923-830671-831072 87 129 R	1300	tpi
2163	YQI_NC003923-378916-379431 142 167 F	440	YQI_NC003923-378916-379431 259 284 R	1076	yqi
2166	YQI_NC003923-378916-379431 275 300 F	219	YQI_NC003923-378916-379431 364 396 R	1013	yqi

[434] The same samples analyzed for drug resistance and virulence in Example 14 were subjected to triangulation genotyping analysis. The primer pairs of Table 21 were used to produce amplification products by PCR, which were subsequently purified and measured by mass spectrometry. Base compositions were calculated from the molecular masses and are shown in Tables 22A and 22B.

Table 22A: Triangulation Genotyping Analysis of Blinded Samples of Various Strains of *Staphylococcus aureus* with Primer Pair Nos. 2146, 2149, 2150 and 2156

Sample Index No.	Strain	Primer Pair No. 2146 (arcC)	Primer Pair No. 2149 (aroE)	Primer Pair No. 2150 (aroE)	Primer Pair No. 2156 (gmK)
CDC0010	COL	A44 G24 C18 T25	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32

CDC0015	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0019	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0026	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0030	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC004	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0014	COL	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC008	????	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC001	Mu50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0022	Mu50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC006	Mu50	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0011	MRS252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0012	MRS252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0021	MRS252	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31
CDC0023	ST:110	A45 G24 C18 T28	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0025	ST:110	A45 G24 C18 T28	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC005	ST:338	A44 G24 C18 T29	A59 G23 C19 T51	A40 G36 C14 T42	A51 G29 C21 T31
CDC0010	ST:338	A44 G24 C18 T29	A59 G23 C19 T51	A40 G36 C14 T42	A51 G29 C21 T31
CDC002	ST:108	A46 G23 C20 T26	A58 G24 C19 T51	A42 G36 C12 T42	A51 G29 C20 T32
CDC0028	ST:108	A46 G23 C20 T26	A58 G24 C19 T51	A42 G36 C12 T42	A51 G29 C20 T32
CDC003	ST:107	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0013	ST:112	ND	A59 G24 C18 T51	A40 G36 C13 T43	A51 G29 C21 T31
CDC0016	ST:120	A45 G23 C18 T29	A58 G24 C19 T51	A40 G37 C13 T42	A51 G29 C21 T31
CDC0027	ST:105	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31
CDC0029	MRS2476	A45 G23 C20 T27	A58 G24 C19 T51	A40 G36 C13 T43	A50 G30 C20 T32
CDC0020	ST:15	A44 G23 C21 T27	A59 G23 C18 T52	A40 G36 C13 T43	A50 G30 C20 T32
CDC0024	ST:137	A45 G23 C20 T27	A57 G25 C19 T51	A40 G36 C13 T43	A51 G29 C22 T30
CDC0031	***	No product	No product	No product	No product

Table 22B: Triangulation Genotyping Analysis of Blinded Samples of Various Strains of *Staphylococcus aureus* with Primer Pair Nos. 2146, 2149, 2150 and 2156

Sample Index No.	Strain	Primer Pair No. 2157 (pta)	Primer Pair No. 2161 (tpi)	Primer Pair No. 2163 (yqi)	Primer Pair No. 2166 (yqi)
CDC0010	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0015	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0019	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
CDC0026	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37

CDC0030	COL	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37
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CDC0020	ST:135	A33 G25 C22 T29	A50 G28 C21 T30	A42 G36 C22 T43	A36 G31 C18 T37
CDC0024	ST:137	A33 G25 C22 T29	A51 G28 C22 T28	A42 G36 C22 T43	A37 G30 C18 T37
CDC0031	***	A34 G25 C25 T25	A51 G27 C24 T27	No product	No product

[435] Note: *** The sample CDC0031 was identified as *Staphylococcus scleiferi* as indicated in Example 14. Thus, the triangulation genotyping primers designed for *Staphylococcus aureus* would generally not be expected to prime and produce amplification products of this organism. Tables 22A and 22B indicate that amplification products are obtained for this organism only with primer pair numbers 2157 and 2161.

[436] A total of thirteen different genotypes of *Staphylococcus aureus* were identified according to the unique combinations of base compositions across the eight different bioagent identifying amplicons obtained with the eight primer pairs. These results indicate that this eight primer pair panel is useful for analysis of unknown or newly emerging strains of *Staphylococcus aureus*. It is expected that a kit

comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Example 16: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for Members of the Bacterial Genus *Vibrio*

[437] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of eight triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within seven different housekeeping genes which are listed in Table 23. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 23.

Table 23: Primer Pairs for Triangulation Genotyping Analysis of Members of the Bacterial Genus *Vibrio*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
1098	RNASEP_VBC_331_349_F	325	RNASEP_VBC_388_414_R	1163	RNase P
2000	CTXB_NC002505_46_70_F	278	CTXB_NC002505_132_162_R	1039	ctxB
2001	FUR_NC002505_87_113_F	465	FUR_NC002505_205_229_R	1037	fur
2011	gyrB_NC002505_1161_1190_F	148	gyrB_NC002505_1255_1284_R	1172	gyrB
2012	OMP NC002505_85_110_F	190	OMP NC002505_154_180_R	1254	ompU
2014	OMP NC002505_431_455_F	266	OMP NC002505_544_567_R	1094	ompU
2323	CTXA_NC002505-1568114-1567341_122_149_F	508	CTXA_NC002505-1568114-1567341_186_214_R	1297	ctxA
2927	GAP NC002505_694_721_F	259	GAP NC_002505_29_58_R	1060	gapA

[438] A group of 50 bacterial isolates containing multiple strains of both environmental and clinical isolates of *Vibrio cholerae*, 9 other *Vibrio* species, and 3 species of Photobacteria were tested using this panel of primer pairs. Base compositions of amplification products obtained with these 8 primer pairs were used to distinguish amongst various species tested, including sub-species differentiation within *Vibrio cholerae* isolates. For instance, the non-O1/non-O139 isolates were clearly resolved from the O1 and the O139 isolates, as were several of the environmental isolates of *Vibrio cholerae* from the clinical isolates.

[439] It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.

Example 17: Selection and Use of Triangulation Genotyping Analysis Primer Pairs for Members of the Bacterial Genus *Pseudomonas*

[440] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by triangulation genotyping analysis, a panel of twelve triangulation genotyping analysis primer pairs was selected. The primer pairs are designed to produce bioagent identifying amplicons within seven different housekeeping genes which are listed in Table 24. The primer sequences are found in Table 2 and are cross-referenced by the primer pair numbers, primer pair names or SEQ ID NOs listed in Table 24.

Table 24: Primer Pairs for Triangulation Genotyping Analysis of Members of the Bacterial Genus *Pseudomonas*

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:1)	Reverse Primer Name	Reverse Primer (SEQ ID NO:1)	Target Gene
2949	ACS_NC002516-970624-971013 299 316 F	376	ACS_NC002516-970624-971013 364 383 R	1265	acsA
2950	ARO_NC002516-26883-27380 4 26 F	267	ARO_NC002516-26883-27380 111 128 R	1341	aroE
2951	ARO_NC002516-26883-27380 356 377 F	705	ARO_NC002516-26883-27380 459 484 R	1056	aroE
2954	GUA_NC002516-4226546-4226174 155 178 F	710	GUA_NC002516-4226546-4226174 265 287 R	1259	guaA
2956	GUA_NC002516-4226546-4226174 242 263 F	374	GUA_NC002516-4226546-4226174 355 371 R	1111	guaA
2957	MUT_NC002516-5551150-5550717 5 26 F	545	MUT_NC002516-5551150-5550717 99 116 R	978	mutL
2959	NUO_NC002516-2984589-2984954 8 26 F	249	NUO_NC002516-2984589-2984954 97 117 R	1095	nucD
2960	NUO_NC002516-2984589-2984954 218 239 F	195	NUO_NC002516-2984589-2984954 301 326 R	1376	nucD
2961	PPS_NC002516-1915014-1915383 44 63 F	311	PPS_NC002516-1915014-1915383 140 165 R	1014	pps
2962	PPS_NC002516-1915014-1915383 240 258 F	365	PPS_NC002516-1915014-1915383 341 360 R	1052	pps
2963	TRP_NC002516-671831-672273 24 42 F	527	TRP_NC002516-671831-672273 131 150 R	1071	trpE
2964	TRP_NC002516-671831-672273 261 282 F	490	TRP_NC002516-671831-672273 362 383 R	1182	trpE

[441] It is expected that a kit comprising one or more of the members of this panel will be a useful embodiment of the present invention.

[442] The present invention includes any combination of the various species and subgeneric groupings falling within the generic disclosure. This invention therefore includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[443] While in accordance with the patent statutes, description of the various embodiments and examples have been provided, the scope of the invention is not to be limited thereto or thereby.

Modifications and alterations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention.

[444] Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims, rather than by the specific examples which have been presented by way of example.

[445] Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank gi or accession numbers, internet web sites, and the like) cited in the present application is incorporated herein by reference in its entirety.

CLAIMS

What is claimed is:

1. An oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 456.
2. An oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 1261.
3. A composition comprising the primer of claim 1.
4. The composition of claim 3 further comprising an oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 1261.
5. The composition of claim 4 wherein either or both of said primers comprises at least one modified nucleobase.
6. The composition of claim 5 wherein said modified nucleobase is 5-propynyluracil or 5-propynylcytosine.
7. The composition of claim 4 wherein either or both of said primers comprises at least one universal nucleobase.
8. The composition of claim 7 wherein said universal nucleobase is inosine.
9. The composition of claim 4 wherein either or both of said primers further comprises a non-templated T residue on the 5'-end.
10. The composition of claim 4 wherein either or both of said primers comprises at least one non-template tag.
11. The composition of claim 4 wherein either or both of said primers comprises at least one molecular mass modifying tag.
12. A kit comprising the composition of claim 4.

13. The kit of claim 12 further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.
14. The kit of claim 12 further comprising one or more calibration polynucleotides.
15. The kit of claim 12 further comprising at least one anion exchange functional group linked to a magnetic bead.
16. A method for identification of a strain of *Staphylococcus aureus* in a sample comprising:
amplifying nucleic acid from said strain of *Staphylococcus aureus* using the composition of claim 4 to obtain an amplification product;
determining the molecular mass of said amplification product;
optionally, determining the base composition of said amplification product from said molecular mass; and
comparing said molecular mass or said base composition with a plurality of molecular masses or base compositions of known amplification products of strains of *Staphylococcus aureus* defined by the composition of claim 4, wherein a match between said molecular mass or base composition and a member of said plurality of molecular masses or base compositions identifies said strain of *Staphylococcus aureus*.
17. The method of claim 16 further comprising repeating said amplifying, determining, optionally determining, and comparing steps using at least one additional primer pair, wherein each member of said at least one additional primer pair is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 288:1269, 698:1420, 217:1167, 399:1041, 430:1321, 174:853, and 172:1360.
18. The method of claim 16 wherein said strain of *Staphylococcus aureus* is a virulent strain.
19. The method of claim 18 wherein said strain of *Staphylococcus aureus* is a virulent strain.
20. A method for determination of the quantity of a strain of *Staphylococcus aureus* in a sample comprising:

contacting said sample with the composition of claim 4 and a known quantity of a calibration polynucleotide comprising a calibration sequence;

concurrently amplifying nucleic acid from said a strain of *Staphylococcus aureus* and nucleic acid from said calibration polynucleotide in said sample with the composition of claim 4 to obtain a first amplification product comprising a bacterial bioagent identifying amplicon and a second amplification product comprising a calibration amplicon;

determining the molecular mass and abundance for said bacterial bioagent identifying amplicon and said calibration amplicon; and

distinguishing said bacterial bioagent identifying amplicon from said calibration amplicon based on molecular mass, wherein comparison of bacterial bioagent identifying amplicon abundance and calibration amplicon abundance indicates the quantity of said strain of *Staphylococcus aureus* in said sample.

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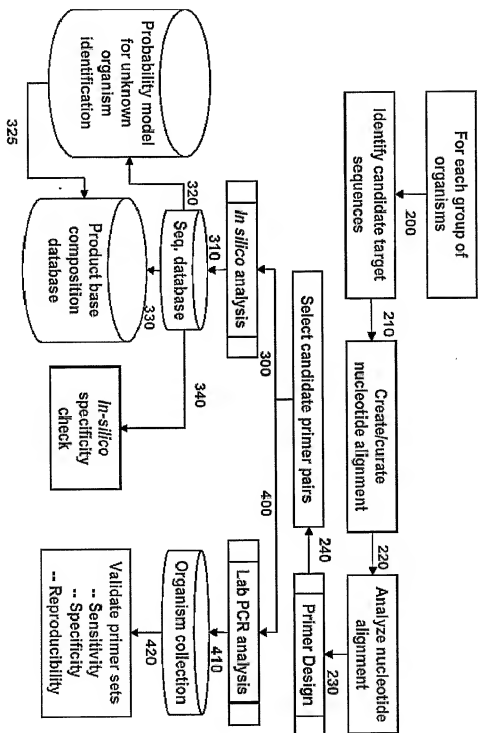


Figure 1

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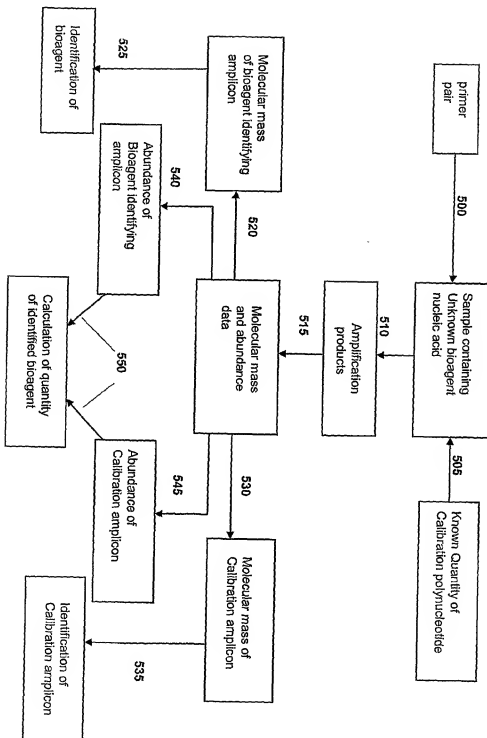
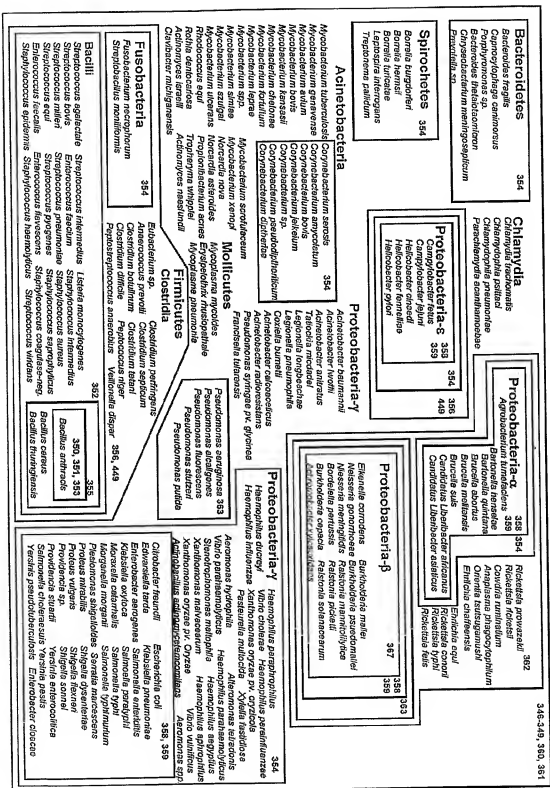


Figure 2



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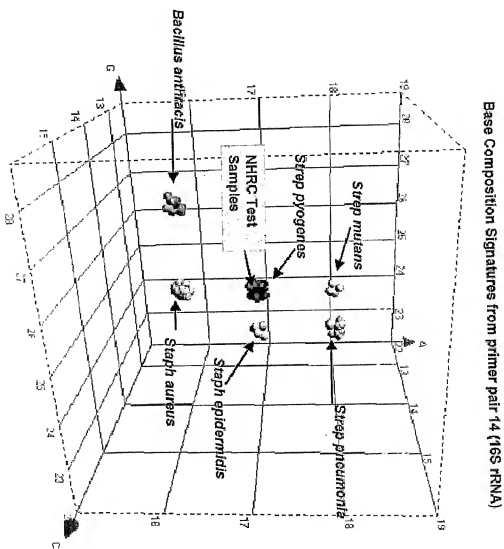


Figure 4

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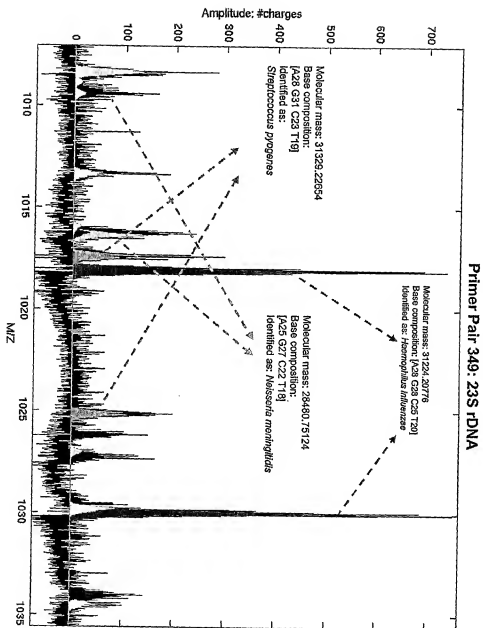
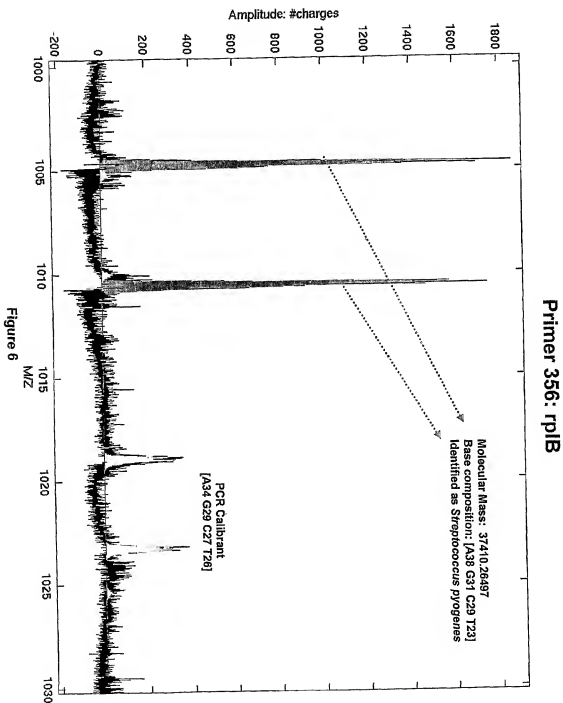


Figure 5

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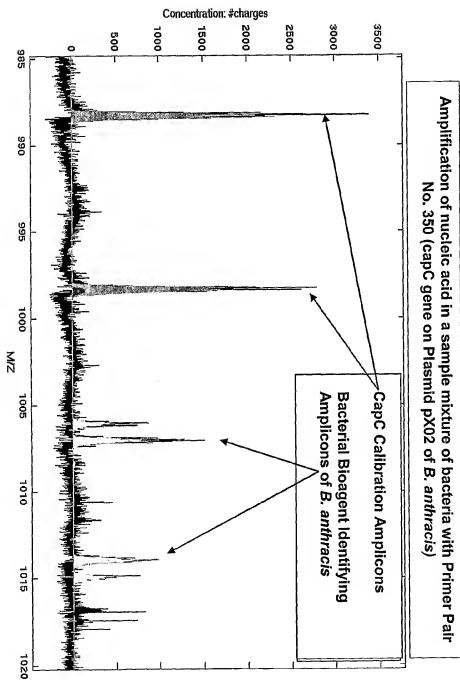


Figure 7

SEQUENCE LISTING

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Hall, Thomas A.
Eshoo, Mark W.

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